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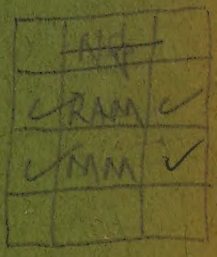

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Titles should be as brief as possible.

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SALMONELLA INFECTION IN CALVES AND OTHER ANIMALS

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(Received 24 August 1961)

ABSTRACT

In a 2½-year survey of salmonella infection in animals being slaughtered at meat works, approximately 5% of 7,497 samples, from 2,755 animals, and the pasture and water supply of five farms, were found to contain salmonellae. The predominant type was *S. typhimurium*, although a few isolations of *S. bovis morbificans*, *S. senftenberg*, *S. newington*, *S. derby*, and *S. anatum* were made. About 15% of the cows and sheep, 13% of the calves, and 4% of the beef cattle were found to have salmonellae in one or more of the samples examined. The principal source of this infection appeared to be on the farm.

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INTRODUCTION

It has been known for some time that salmonellosis is widespread among animals throughout the world (Buxton 1957), and this problem has received considerable attention in many countries. The importance of this disease in animals in New Zealand has been recognised for many years. In a recent publication Salisbury (1958) has reported the isolation of salmonellae from nearly 1,400 animals and birds which showed obvious symptoms of disease. Of 416 isolations from cattle and 243 from sheep, all but a few were identified as *S. typhimurium*. *S. cholerae-suis* was found in 407 of 416 isolations from pigs and *S. pullorum* in 165 of 173 isolations from poultry.

Until recently most attention was paid to the disease as it affected the animal, and the possibility of sub-clinical infections or the carrier state in animals, giving rise to the spread of organisms of the salmonella group in the products from those animals, was ignored. Studies of infections in diseased animals may not always give an accurate indication of the incidence of infection in food-stuffs produced from apparently healthy animals. In the past few years, however, the search for the sources of salmonellae infecting food-stuffs has led to a number of surveys of salmonella infection in animals being slaughtered for food (Anderson *et al.* 1961; Galton *et al.* 1954; Josland 1950; Murdock and Gordon 1953; Ritchie and Clayton 1951; Report 1955).

In the present investigation a survey was made, over a period of 2½ years, of the incidence of salmonella infection in apparently healthy animals being slaughtered at meat works. Faeces samples from cows affected by a variety of disorders were also examined.

EXPERIMENTAL

Collection of samples

Four types of sample—faeces, liver, spleen, and mesenteric lymph nodes—were collected from the majority of the animals examined in this survey. The liver, spleen, mesenteric lymph nodes, and a portion of the gut near the anus were all removed together, as soon as possible after slaughter, and placed on a sterile plastic tray. The faeces samples were obtained by squeezing the gut so as to expel the faeces into a plastic bag. A sterile knife was used to remove the mesenteric lymph nodes and a portion of the liver and spleen, and these samples were also placed in individual plastic bags. The samples were stored at about 4°c until examination, which was usually carried out within 10 days of collection.

At one stage during this investigation, August to October 1958, a proportion of the samples collected each week was frozen and stored in the frozen state for up to two months before examination. It was noted that very few of the frozen samples appeared to contain salmonellae although other samples collected at the same time showed a high incidence of infection. Experiments on the effect of freezing inoculated samples of meat showed a three-fold

decrease in numbers of salmonellae after freezing and storage for one week, with a further four-fold decrease after six weeks' storage. As the effect of freezing may be more significant when the numbers of salmonellae are very low the use of frozen samples was abandoned.

The faeces samples from sick cows were taken by the veterinarian attending the animal and were placed in McCartney bottles containing 10 ml of buffered glycerol preservative medium (Mackie and McCartney 1956) for transport to the laboratory. The preservative medium was used also for transport of rectal swabs obtained from cows.

Examination of samples

Approximately 1 g of faeces and 5 g of the other samples were placed in 8 oz screw-capped jars with 100 ml of selective enrichment medium. Tissue samples were shredded with sterile scissors before addition of the medium. The samples were prepared in duplicate, and both selenite broth (DIFCO) and tetrathionate broth (DIFCO) were used for enrichment. SS (DIFCO) and Wilson and Blair, bismuth sulphite agar plates (DIFCO) were inoculated by loop from both the enrichment broths after 24 and 72 hours' incubation at 37°c, thus giving eight plates per sample.

After 48 hours' incubation at 37°c the plates were examined and two to four salmonella-like colonies picked off and inoculated into tubes of McConkey broth and Gillies (1956) media. Cultures which gave the typical salmonella reaction in these media were tested with salmonella sera (Standards Laboratory for Serological Reagents, Colindale, London). The identification of all the salmonella isolations was confirmed by the National Health Institute, Wellington, New Zealand. All *S. typhimurium* cultures were forwarded to the Central Enteric Reference Laboratory, Colindale, for phage typing.

RESULTS

During this investigation a total of 7,497 samples from 2,755 animals and the soil and water supplies of five farms was examined. Of this total, 344 samples from 224 animals and 11 of 80 soil samples were found to contain organisms of the salmonella group. Details of the results showing the incidence of infection in each type of sample are given in Table 1. The table includes the results from only those animals from which a full set of the four types of sample —faeces, liver, spleen, and mesenteric lymph nodes—was obtained.

Nineteen samples from 11 of the calves and 11 samples from 7 of the cows contained *S. bovis morbificans,* 5 samples from 4 of the calves *S. anatum,* and one cow sample *S. newington.* The remaining 326 isolates were identified as various phage types of *S. typhimurium,* details of which are given in Table 2. A number of cases of multiple infection was observed. In seven of these cases two types of salmonella were isolated from one sample. Two calves and five cows were

TABLE 1. SALMONELLA-INFECTED SAMPLES

Type of sample	Number examined	Number positive	% positive
Calf Faeces	1,110	70	6.3
Liver	1,110	48	4.3
Spleen	1,110	84	7.6
Mesenteric Lymph Nodes	1,110	39	3.5
Total Calves	1,110	140	12.6
Cow Faeces	133	16	12.0
Liver	133	3	2.3
Spleen	133	3	2.3
Mesenteric Lymph Nodes	133	11	8.3
Total Cows	133	20	15.0
Beef Cattle Faeces	48	1	2.1
Liver	48	1	2.1
Spleen	48	0	0
Mesenteric Lymph Nodes	48	2	4.2
Total Beef Cattle	48	2	4.2
Sheep Faeces	33	1	3.0
Liver	33	0	0
Spleen	33	1	3.0
Mesenteric Lymph Nodes	33	3	9.1
Total Sheep	33	5	15.2

TABLE 2. *Salmonella typhimurium* PHAGE TYPES

Phage type	Number of isolates
1	245
1 var 1	6
1 var 2	15
1 var 4	19
1 var 5	3
1 a	1
1 a var 2	2
Group 1	2
2	11
2 b var 1	1
U42	7
U56	1
12	2
16	6
Untypable	5
Total	326

infected with *S. bovis morbificans* as well as *S. typhimurium*. The mesenteric lymph nodes of one cow contained both *S. typhimurium* and *S. newington*, and the faeces of one calf contained *S. anatum*, with *S. bovis morbificans* being isolated from both the liver and mesenteric lymph nodes. Although only one culture of *S. typhimurium* from each sample was phage typed, five calves and three cows had two phage types of *S. typhimurium* present in different samples. Samples from two of these cows contained *S. bovis morbificans* in addition to the two phage types of *S. typhimurium*.

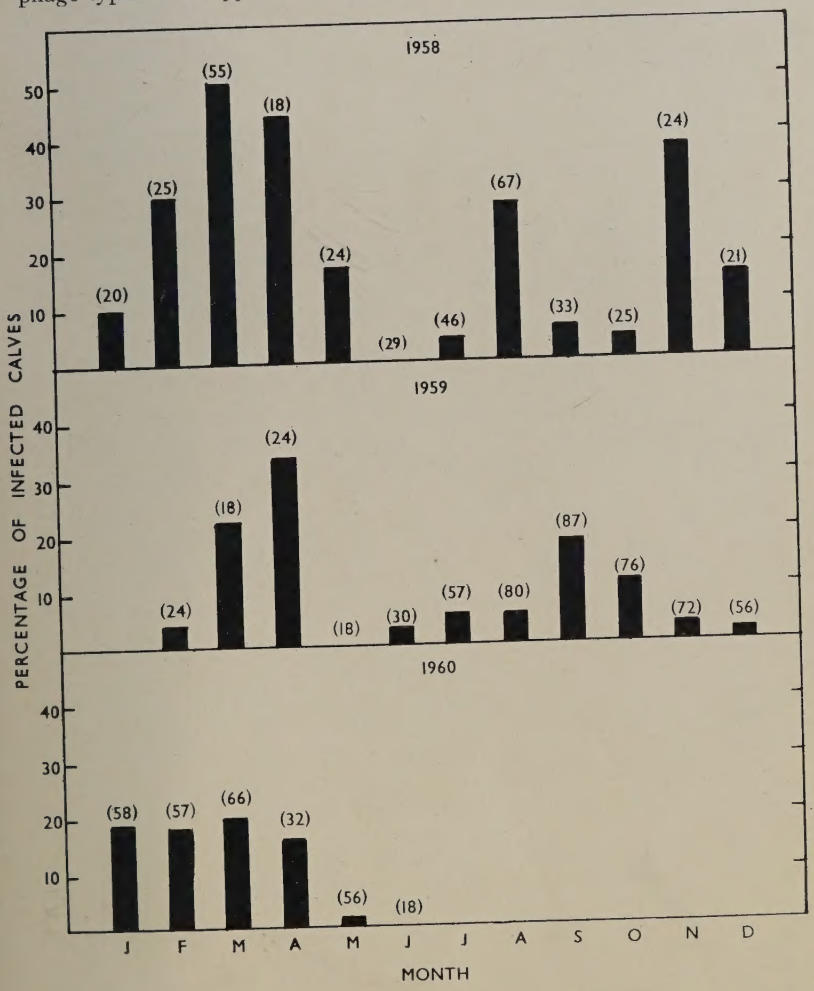

Fig. 1.—Seasonal variation of salmonella infection in calves. (Numbers in parenthesis indicate number of calves examined each month.).

An animal was regarded as being infected if salmonellae were isolated from one or more of the four samples examined. Over 50% were found to be infected in one sample only and less than 10% in all four samples. The distribution of salmonellae in samples from the infected calves and cows is shown in Table 3.

The survey of infection in calves was carried out over a period of 2½ years, from January 1958 to June 1960, and during this time a marked seasonal variation in the incidence of infection was observed. The percentage of infected calves among those examined each month is shown in Fig. 1. For the first three months of this period the results include calves from which only faeces samples were examined. For these animals the percentage infected has been calculated on the basis that only 50% of infected calves show positive faeces (Table 3). Cow samples were examined in 11 months of this period. In general, the seasonal variations were similar to those for calves.

TABLE 3. DISTRIBUTION OF SALMONELLA-POSITIVE SAMPLES FROM INFECED ANIMALS

		Calves (140) No. positive	%	Cows (20) No. positive	%
Total number of animals positive in	Faeces	70	50	16	80
	Liver	48	34	3	15
	Spleen	84	60	3	15
	Mesenteric Lymph Nodes	39	28	11	55
	All samples	12	9	1	5
Animals positive in one sample only	Faeces	23	16	8	40
	Liver	12	9	0	—
	Spleen	36	26	0	—
	Mesenteric Lymph Nodes	9	6	3	15
	Total	80	57	11	55
Total number of animals with salmonella in	Faeces and/or spleen	119	85	17	85
	Faeces and/or mesenteric lymph nodes	82	59	20	100

The effect of transportation and holding times on the incidence of infection in calves was studied. The calves examined could be divided into three more or less equal groups for which: (1) the transit time was 12 hours or more; (2) the holding time was more than 12 hours; and (3) the combined transportation and holding time was less than 12 hours. The incidence of infection in the first two groups was about 14% compared with an incidence of about 10% in the third group.

In an attempt to trace the source of infection, rectal swabs from animals, and samples of soil, drainage, and water supplies from farms known to have supplied infected animals, were examined. Although no salmonellae were found in rectal swabs from 209 cows, yearlings, and calves, and 15 samples of drinking water, *S. typhimurium* was present in 11 of 80 samples of soil and drainage. The majority of the positive soil samples came from one farm where 10 of 20 soil and drainage samples contained *S. typhimurium* (types 1 and U42). During the previous nine months at least four cows on this farm had developed acute, although not fatal, salmonellosis. The last case occurred within a month of the collection of the soil and drainage samples, and as salmonellae can survive for long periods in soil and pasture (Josland 1951) it was not surprising to find such a high incidence of infection around the farm. All the cows on this farm were sampled by rectal swab about three weeks after the examination of the soil samples. Although no salmonellae were found in these samples, the possibility of one or more of the cows being an intermittent excretor of salmonellae cannot be ruled out.

To test the hypothesis that other infections or metabolic disorders predispose animals to salmonella infection, faeces samples from 104 dairy cows diagnosed as suffering from a variety of disorders were examined. *S. typhimurium* was isolated from 12 of these samples.

DISCUSSION

In view of earlier work on the incidence of salmonella infection in animals in New Zealand, particularly in connection with calves, it was surprising to find such a high incidence of infection in apparently healthy animals. Josland (1950), in a survey of the incidence of salmonella infection in cattle—some of which came from the areas covered by the present survey—recorded the isolation of *S. typhimurium* from only about 0.4% of intestinal lymph nodes from 2,654 cattle. In an investigation into the causes of calf scours, Osborne and Ensor (1954) found only four salmonella-infected calves out of a total of 302 examined, and it has been claimed that salmonellae are not important pathogens in young cattle in this country (Salisbury 1958). The results of the present investigation show that 12.6% of the calves and 15.0% of the cows examined carried salmonella organisms in their faeces or organs. These results suggest that, even though the reported incidence of salmonellosis may not be high, the infection is widespread.

The high incidence of infection in calves is of interest in view of the conflicting opinions regarding the susceptibility of young animals to salmonella infection. Jepson of Copenhagen and several other continental veterinarians (N. H. Law pers. comm.) claim that the calf is immune to salmonella infection for the first two to three weeks of life. Field (1948), on the other hand, is of the opinion that the infection can be transmitted from cow to calf at birth, and also that the very young calf is particularly susceptible to infection from other sources, especially when it is in a weakened condition from lack of food, stress of travel, and other factors.

The discrepancy between the results of this and earlier investigations may be due to seasonal variations or to differences in the methods of sampling employed. It will be seen from Fig. 1 that if sampling had been restricted to certain periods during this time the results would have differed greatly from those obtained for the whole period. The incidence of infection appears to differ quite widely from year to year. In 1958 nearly 19% of the calves examined were found to be infected, whereas in 1959 this figure was about 9%. The results for the first half of 1960 showed an incidence of 14%, about midway between the figures for the corresponding period in the previous two years. In many of the earlier surveys, both in New Zealand and overseas, sampling was restricted to one or two types of samples. For example, Josland (1950) examined only bile and intestinal lymph nodes, and Osborne and Ensor (1954) studied faeces samples only. In surveys carried out by the British Public Health Laboratory Service, and in other surveys carried out overseas, the most common type of sample examined was faeces.

As shown in Table 3, salmonellae were isolated more frequently from calf spleen and faeces than from the other two types of samples, and if examination had been confined solely to faeces and spleen samples 85% of the infected calves would have been detected. The distribution of salmonellae in cow samples differed from that in calf samples. Here, all the positives would have been detected if only faeces and mesenteric lymph node samples had been examined. However, it is apparent from Table 3 that if only one type of sample had been examined a high proportion of the infected animals would not have been detected.

It might be considered that the use of two enrichment media and two plating media, as well as plating after 24 and 72 hours' enrichment, involves a lot of unnecessary work. However, it was found that the use of several different media does increase the chances of detecting small numbers of salmonellae in the types of sample examined. Enrichment in tetrathionate broth followed by plating on SS agar proved to be the most successful combination of media. Salmonellae were isolated in this way from 71% of the infected samples, whereas enrichment in selenite broth followed by plating on SS agar gave only 55% recovery. Wilson and Blair plates from tetrathionate and selenite enrichment showed salmonellae in 49% and 42% respectively of the infected samples. If selenite enrichment or plating on Wilson and Blair medium had been omitted, 22% or 13%, respectively, of the infected samples would have been missed. About 9% of the infected samples were detected only by plating after enrichment for 72 hours.

No counts were made of the number of salmonellae in the samples examined, but it appears that in the majority of cases the numbers were relatively low. From results with meat to which known numbers of salmonellae had been added it appears that above about 50 salmonellae per 10 g sample all four plates, inoculated after 24 hours' enrichment, are usually positive. With

less than this number it is rare for all plates to show salmonellae. In the present survey only about 12% of the infected samples were positive on four plates and an additional 14% on three plates.

The predominant type of salmonella isolated from animal samples was *S. typhimurium,* with about 10% of *S. bovis morbificans,* and a few isolations of *S. anatum* and *S. newington.* This is what would be expected from previous work on clinically affected animals in New Zealand (Salisbury 1958). *S. dublin,* which is the principal type of salmonella encountered in cattle in Britain, was not found in the present investigation, and so far has only been isolated from human sources in New Zealand (Salisbury 1958). *S. anatum* has recently been isolated from a pig and a lamb (Davis and Russell 1960), but it has not been reported before in cattle. A few isolations of *S. senftenberg* and *S. derby* were made from effluents from the works where the animals were killed but these organisms were not found in animal samples.

Phage typing of the *S. typhimurium* cultures showed a predominance of type 1, with type 1 var 4, type 1 var 2, and type 2 being the most common types. Type 1 var 4 suddenly appeared in animals from a number of districts, and all 19 cultures of this type were isolated within a period of about one month. During this period nearly 50% of all isolations were identified as type 1 var 4.

It is fortunate that the incidence of infection in calves is at its lowest during the first part of the main calving season and at its highest at the time when the number of calves being slaughtered is relatively low. The reasons for the observed seasonal variation are obscure. This variation does not appear to be directly related to the reported incidence of salmonellosis in adult animals, to climatic conditions, or to volume of kill. Salisbury (1958) has related the incidence of salmonellosis in dairy cattle to stress during the period of maximum butterfat production, with the maximum in both cases coming during October and November. As the calf samples were obtained from very young animals it is possible that the seasonal variation is related to time of calving. The late summer and autumn peak may be due to stress of calving at a time far removed from the more common calving period. It is, however, worth noting that the incidence in cows followed the same general trend as in calves, and here the incidence is not necessarily related to calving time. Owing to the small number of sheep and beef cattle samples examined, it was not possible to observe any seasonal variation in the incidence of infection in these animals.

Although salmonellosis in sheep has been considered to be primarily a disease of the adult and usually of ewes in very fat condition (Salisbury 1958), the five infected sheep detected in the present survey were all under two years of age. Close contact between animals and a sudden reduction in the level of nutrition often lead to outbreaks of salmonellosis in sheep and other animals. This is commonly observed when sheep are held at very high stocking rates, such as are employed in New Zealand as a precautionary measure against the development of facial eczema.

As shown by the work of Anderson *et al.* (1961), the spread of infection during the holding of stock at collecting centres can have a considerable effect on the incidence of infection at the time of slaughter. Although the majority of calves in the meat works from which the samples were obtained are killed within four hours of arrival, some are held for 12 to 24 hours before slaughter. Calves from districts remote from the works may be in transit for up to 20 hours, but as a general rule these animals are slaughtered soon after arrival. Thus the combined transportation and holding times for the calves examined were less than the holding times for the calves studied by Anderson *et al.* (1961). Although, probably because of this, the observed effect of transportation and holding times was less striking, it did confirm the findings of these workers. The incidence of infection in calves which had spent more than 12 hours in transit or holding was about 40% higher than in those which were killed within 12 hours of leaving the farm. This shows the necessity of reducing to a minimum the time taken in transportation and holding at the farm or meat works, and emphasises the importance of maintaining a high standard of hygiene in transport and holding facilities.

Knives, equipment, and water used in the meat works were sampled in a search for possible sources of infection. Although some salmonella contamination was found, it appeared that this was a consequence of handling infected animals and not the primary source of the infection. The observation that about 22% of the infected animals were slaughtered within two hours of arrival at the works suggests that the infection was present before these animals reached the meat works. Liver or spleen samples from 11 calves which had been killed within three hours of leaving the farm were found to contain salmonellae. It is unlikely that the infection could have reached these organs in the time available if the animals had not become infected until after leaving the farm. From the number of different types of salmonellae found (*S. bovis morbificans, S. anatum, S. newington*, and at least 15 phage types of *S. typhimurium*) it appears that the infection had come from a number of sources. Thus, although the infection can spread during the transportation and holding of stock, the most likely origin is on the farms from which the animals were obtained.

It was not possible to identify the farms supplying infected animals except in the few instances where the farmer had delivered the stock directly to the meat works. Because of this, only five farms were investigated in an attempt to trace the source of infection. On the farm, calves can become infected through contact with infected cows or indirectly through contamination of pasture and water supplies. Although no salmonellae were found on rectal swabs taken from cows on these farms, *S. typhimurium* was isolated from soil samples from two of the five farms.

On other farms samples of faeces were taken by veterinarians from cows which were affected by a variety of disorders. Of these, about 12% contained *S. typhimurium*. Although the majority of these infected animals showed signs of dysentery, in at least two cases the

diagnosis of the primary disease was other than salmonellosis. That apparently healthy animals, too, can carry the infection is shown by the observation that 15% of the cows examined at the works were symptomless carriers. It would appear, therefore, that the incidence of infection among dairy cows is higher than would be expected from diagnostic reports.

The widespread reservoir of infection in dairy herds not only results in a high incidence of infection in adult animals coming forward for slaughter, but also provides a likely source of the infection in calves.

To reduce the incidence of infection in domestic animals it is necessary to detect and eliminate the carriers. This is likely to be a difficult process. Josland (1953) has stated that the examination by enrichment methods of faeces samples is the only reliable method for the identification of salmonella carriers. However, this investigation has shown that salmonellae may be excreted only intermittently by some carriers, so that a large number of samples will be needed to ensure the detection of all the carriers in any one group of animals. The significant point is that salmonella carriers are a threat, not only to animal health, but also to human health, and efforts should be made, therefore, to break the cycle of infection and institute some form of control.

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REFERENCES

Anderson, E. S.; Galbraith, N. S.; Taylor, C. E. D. 1961: *Lancet 1:* 854–8.

Buxton, A. 1957: "Salmonellosis in animals". pp. 45–98. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks. 209 pp.

Davis, E. A.; Russell, R. R. 1960: *N.Z. Vet. J. 8:* 116–7.

Field, H. I. 1948: *Vet. J. 104:* 251–66, 294–302, 323–39.

Galton, M. M.; Smith, W. V.; McElrath, H. B.; Hardy, A. B. 1954: *J. infect. Dis. 95:* 236–45.

Gillies, R. R. 1956: *J. clin. Path. 9:* 368–71.

Josland, S. W. 1950: *Aust. vet. J. 26:* 249–53.

————1951: *Ibid. 27:* 264–6

————1953: *N.Z. Vet. J. 1:* 131–6.

Mackie, T. J.; McCartney, J. E. 1956: "Handbook of practical bacteriology". 9th ed. p. 320. E. & S. Livingstone, London. 744 pp.

Murdock, C. R.; Gordon, W. A. M. 1953: *Mon. Bull. Minist. Hlth Lab. Serv. 12:* 72–6.

Osborne, H. G.; Ensor, C. R. 1954: *N.Z. Vet. J. 2:* 14–6..

Report 1955: *Mon. Bull. Minist. Hlth Lab. Serv. 14:* 132–8.

Ritchie, J. M.; Clayton, N. M. 1951: *Ibid. 10:* 272–7.

Salisbury, R. M. 1958: *N.Z. Vet. J. 6:* 76–86.

A COMPARISON OF DDT RESIDUES ON PASTURE FOLLOWING TREATMENT WITH DDT PRILLS AND DDT/SUPERPHOSPHATE

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(Received 20 November 1961)

ABSTRACT

Residues of pp′-DDT on pasture were found to be markedly less after pp′-DDT was applied as DDT prills than following its application, in a similar quantity, as DDT/superphosphate dry mix.

Trial plots of pasture were treated with either DDT prills or DDT/superphosphate dry mix at application rates of 1.75 lb or 1.90 lb pp′-DDT per acre respectively, and the pp′-DDT residues on the grass were determined at intervals up to 40 days after treatment. During this time the residues from the prill treatment ranged from 4.9 p.p.m. to 1.0 p.p.m. of the "green" weight of the pasture, while those from the DDT/superphosphate gave immediately following treatment a mean of 67.0 p.p.m. ("green" weight), which decreased to 8.4 p.p.m. at the 40th day.

INTRODUCTION

DDT has been increasingly used in New Zealand during the last 15 years for control of the common grass-grub (*Costelytra* spp.) and subterranean caterpillar (*Oxycanus* spp.) in pasture, and recommendations for control of these insects by DDT were made by Kelsey (1955). DDT has usually been applied mixed with superphosphate.

DDT adheres to pasture, and it is well established that DDT ingested by animals is stored in fat and secreted in milk. Thus Gannon and Decker (1960) showed that cows grazing on pasture that contained 58.0 p.p.m., 28.5 p.p.m., and 6.7 p.p.m. DDT on the 3rd, 7th, and 19th day after treatment respectively gave milk containing a maximum concentration of DDT of 7 to 8 p.p.m. (within 3 to 7 days), and this declined to 2.35 p.p.m. after 20 days.

The amount of DDT retained on pasture depends on the method of application of DDT, the height and type of pasture, and whether it is wet or dry at the time of treatment.

There is a nil tolerance in New Zealand for DDT in dairy products, and it is therefore important to develop methods of application that will result in low residues on pasture. The present investigation was undertaken to compare the magnitude of DDT residues on pasture after treatment with DDT prills, on the one hand, with DDT/superphosphate dry mix, on the other.

DDT prills are made from fine particles of micronised DDT wettable powder bonded on to an inert calcite core. During application to the pasture the DDT powder is held securely to the core, but following application the fine particles of DDT are released by rain and dispersed around the grass roots.

EXPERIMENTAL PROCEDURE AND RESULTS

The experimental area was situated at Mapua, Waimea County, Nelson, and was in established pasture, predominantly ryegrass and clover with some cocksfoot. The area was close mown and top-dressed with urea on 5 April 1961, and fenced free of grazing stock. On 3 May 1961, when the grass was about 3 in. high, six plots, each one-eightieth of an acre in area, were delimited by mowing 2 ft guard strips from which a sample of grass for control analysis was taken. Three replicates of each of the two treatments (DDT prills or DDT/superphosphate) were then applied. Throughout the period of application (1.30 p.m. to 2.30 p.m.) the weather was fine and almost calm and there was no moisture visible on the pasture. The water content of the grass was 80 per cent.

The prills had a median size of 16 British Standard sieve, and ranged from 2 per cent retained on 10 mesh to 99 per cent on 25 mesh.

Prills containing 8.65 per cent pp′–DDT were applied at the rate of 20 lb per acre, viz 4 oz per plot or 1.75 lb pp′–DDT per acre. To ensure even distribution the 4 oz of DDT prills were diluted with 36 oz of clay-coated prills, and each plot was divided into four sections. To each section 10 oz of diluted prills and 11.2 oz of straight superphosphate were applied.

DDT/superphosphate was prepared by the addition of 22.4 g of 48.5 per cent pp′–DDT to 44.8 oz of superphosphate, giving a mixture containing the equivalent of 1.90 lb of pp′–DDT to each 2 cwt of superphosphate. This was applied at the rate of 1.90 lb pp′–DDT per acre, viz 45.6 oz of DDT/superphosphate mix per plot. Each plot was divided into four sections, and 11.4 oz of the DDT/superphosphate was applied to each section.

Samples of grass were cut 30 min. after treatment and on the 14th and 40th day after treatment. Approximately one third of each plot was hand mown at each sampling to a stubble height of approximately 1 in., care being taken to avoid the edges of the plots and areas previously mown. The length of grass at the first, second, and third cuts was 3 in., 4 in., and 6 in. respectively. A

sample of 1 to 2 lb taken from the grass cut from each plot was sealed in a polythene bag, sent within one day to Dominion Laboratory, D.S.I.R., Wellington, and stored at 4–5°c until analysed. Daily records of rainfall were kept throughout the trial.

The grass was finely chopped and mixed thoroughly, and duplicate 100 g subsamples from each plot sample were taken for determination of pp′–DDT. At the same time a 5 g sample was taken for determination of moisture by the method of Dean and Stark.

The 100 g sample of grass was placed in a 4 pint preserving jar and wetted thoroughly with 150 ml of "Analar" grade isopropanol. Four hundred millilitres of redistilled hexane were added and the jars were covered with a double thickness of polythene sheet, sealed, and shaken for 15 min. on a mechanical shaker. A quantity of 750 ml of distilled water was added, and the shaking was continued for an additional 30 min.

A known volume (*ca.* 300 ml) of the hexane layer was drawn off through a No. 2 sintered glass filter stick into a 500 ml graduated cylinder, the clear green solution was concentrated in a 500 ml Kudema-Danish evaporator fitted with a 3 ball Snyder column, and the last few millilitres of solvent were evaporated with a jet of air. The residue dissolved in *ca.* 15 ml of warm carbon tetrachloride was added to a column (37 mm × 310 mm) containing (from bottom to top) 20 g of acid-celite (Davidow 1950), a 25 mm layer of anhydrous sodium sulphate, 25 g of chromatographic alumina, and a 30 mm layer of anhydrous sodium sulphate. (D. L. Harrison pers. comm.). Carbon tetrachloride was used as eluting solvent. The first 20 ml of eluate were rejected, then 250 ml were collected. An aliquot (25 ml to 100 ml) was transferred to a tube with standard joint and concentrated in a jet of air at 60–70°c to *ca.* 2 ml and finally evaporated to dryness at room temperature.

The procedure then followed was that of Palmer-Jones *et al.* (1954), based on the nitration method of Schechter *et al.* (1945). The final colorimetric determination was carried out using 10 ± 0.1 per cent sodium methoxide. The optical density at 600 mμ was measured 15 min. after the addition of the sodium methoxide, using a 1 cm cell and a Beckman Model DU spectrophotometer. Readings at 450 mμ were also taken as a check on extraneous red and yellow colours and, if considered too high, another determination was carried out.

The use of ethanolic potassium hydroxide as chromogenic agent (Sergeant and Wood 1959) in place of sodium methoxide was found to give a less stable colour.

The apparent pp′–DDT content in the control grass gave a mean of 0.5 p.p.m. of the "green" weight (2.9 p.p.m. of the dry weight).

When known amounts of pp′–DDT were added to control samples of grass before extraction, the recovery was 93 per cent.

TABLE 1. DDT RESIDUES ON PASTURE AFTER TREATMENT WITH DDT PRILLS OR DDT/SUPERPHOSPHATE

Treatment	Rate of application (lb pp′-DDT/acre)	Interval between application and cutting dates	pp′-DDT dry wt. basis p.p.m.		pp′-DDT "green" wt. basis p.p.m.	
Control			2.9*		0.5*	
Prill	1.75	30 minutes	10.5	12.5	1.9	2.3
"	"	" "	12.5	14.0	2.3	2.6
"	"	" "	13.5	11.5	2.5	2.1
DDT/Superphosphate	1.90	" "	355	305	64.5	55.5
" "	"	" "	345	370	63.0	67.5
" "	"	" "	405	420	73.5	76.5
Prill	1.75	14 days	26.5	27.0	4.8	4.9
"	"	" "	16.0	11.0	2.9	2.0
"	"	" "	19.0	15.0	3.5	2.7
DDT/Superphosphate	1.90	" "	110	100	20.0	18.0
" "	"	" "	180	165	33.0	30.0
" "	"	" "	185	185	33.5	33.5
Prill	1.75	40 days	7.2	6.7	1.3	1.2
"	"	" "	5.9	6.1	1.1	1.1
"	"	" "	5.8	5.7	1.1	1.0
DDT/Superphosphate	1.90	" "	44.5	45.5	8.1	8.3
" "	"	" "	53.5	52.0	9.7	9.5
" "	"	" "	38.0	42.0	6.9	7.6

* Mean of 5 determinations.

Rainfall of 3.37 in. fell between first cut and second cut at 14 days and a further 1.38 in. before taking samples at 40 days.

The results (see Table 1) are expressed in p.p.m. pp′–DDT on a dry weight basis and on a "green" weight basis (recalculated to a common moisture content of 81.8 per cent), and are not corrected for the apparent DDT content in the control grass and for the recovery.

DISCUSSION

The residues from the prills gave a mean value of 2.3 p.p.m. of the "green" weight 30 min. after treatment, which increased to 3.5 p.p.m. 14 days later, and then dropped to 1.1 p.p.m. on the 40th day. Residues from the DDT/superphosphate mix gave, immediately following treatment, a mean of 67.0 p.p.m. of the "green" weight, which fell to 28.0 p.p.m. and finally 8.4 p.p.m. This decrease in DDT residue from the DDT/superphosphate treatment would be partly accounted for by the dilution effect of the 3 in. growth of grass. Despite the heavy rainfall of 3.37 in. (3.29 in. over 3 days) between the 1st and 2nd samplings, only about half of the DDT/superphosphate was washed off the grass. The residue of DDT from the DDT prill treatment is higher after 14 days than after 30 min. However, the first cut (at 30 min.) was taken from the DDT prill plots before the DDT/superphosphate treatment was applied to the adjoining plots. The reason for the higher residues for prills at 14 days is not known but could only be due to drift of DDT from the DDT/superphosphate plots or to rain splashing DDT from the prills on the soil surface up on to the pasture.

The results indicate that the amount of pp′–DDT residue on pasture was markedly less when pp′–DDT was applied as prills than when applied, in a similar quantity, as DDT/superphosphate dry mix.

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REFERENCES

Davidow, B. 1950: Isolation of DDT from fats. *J. Ass. off. agric. Chem. Wash. 33:* 130–2.

Gannon, N.; Decker, G. C. 1960: The excretion of dieldrin, DDT, and heptachlor epoxide in milk of dairy cows fed on pastures treated with dieldrin, DDT, and heptachlor. *J. econ. Ent. 53:* 411–5.

Kelsey, J. M. 1955: Control of grass-grub and subterranean caterpillar. *N.Z. Dep. sci. industr. Res. Ent. Sta. Publ. 14. (2nd revision).* 19 pp.

Palmer-Jones, T.; Bartrum, F. A.; Forster, I. W.; Harrison, D. L. 1954: Effect on honey bees of DDT plus superphosphate applied as a dust to white clover pasture. *N.Z. J. Sci. Tech. A36:* 177–92.

Schechter, M. S.; Soloway, S. B.; Hayes, R. A.; Haller, H. L. 1945: Colorimetric determination of DDT. Color test for related compounds. *Industr. Engng Chem. (Anal.) 17:* 704–9.

Sergeant, G. A.; Wood, R. 1959: The determination of DDT residues in foodstuffs. *Analyst 84:* 423–6.

PRELIMINARY INVESTIGATIONS ON THE PENETRATION AND PERSISTENCE OF DDT UNDER PASTURE

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ABSTRACT

DDT is applied to pasture in conjunction with superphosphate, for the control of damage by soil insects, particularly grass-grub (*Costelytra*). In a plot trial on Papatoetoe silt loam, DDT penetrated to a maximum depth three months after application. Most of the insecticide recovered was associated with the top 2 in. of soil. Decomposition of DDT appeared to take place solely within the top 1 in. of soil, approximately 50% loss being observed in the initial 12-month period of the trial. No significant difference in distribution pattern or breakdown rate could be detected between rates of application ranging from 1 to 8 lb pp′–DDT per acre.

INTRODUCTION

Application of DDT to pasture infested with soil insects, such as grass-grub (*Costelytra*), has proved to be the most effective method of control investigated to date. Work in this field was pioneered by Dumbleton *et al.* (1948) and Kelsey (1951, 1955) of the Entomology Division, N.Z. Department of Scientific and Industrial Research.

DDT is most conveniently applied for this purpose in conjunction with routine fertiliser top-dressing. The recommended application rate of 2 lb pp′–DDT per acre is stated to give control for a period of three years. Soil samples from several areas exhibiting marginal grub damage have been analysed by the authors. Under undamaged pasture, the residual pp′–DDT level was invariably greater than 0.6 lb per acre. No diminution in grub activity could be observed at levels below 0.3 lb per acre. J. M. Kelsey (pers. comm.) estimates the minimal residual pp′–DDT level needed to give a measure of protection as approximately 0.5 lb per acre.

In general the results have been satisfactory using this method of application, but in some areas the period of control has been shorter than the claimed three years. Assuming satisfactory initial distribution of the DDT–fertiliser mixture, loss of control could be due either to decomposition of DDT from chemical or bacterial action in the soil, or to migration of DDT away from the zone of grub activity by leaching or mechanical transfer by earth worms. Differences in soil type and climatic conditions may be major contributing factors.

The current extensive use of chlorinated hydrocarbon dust and spray formulations for orchard and crop pest control has prompted overseas research into the accumulation of residues in the soil, and the possible deleterious effects on subsequent crops. Chisholm *et al.* (1950), Ginsberg and Reed (1954), and Ginsberg (1955) have found most of the DDT recovered from a treated soil to be close to the surface, with little evidence of downward distribution except as a result of cultivation. DDT has been found to be the most persistent in soil of the chlorinated hydrocarbon pesticides in common use (Lichtenstein and Schulz 1959). Persistence varied considerably in different soil types, as shown in the work of Lichtenstein (1957), Allen *et al.* (1954), and Boswell *et al.* (1955). Lichtenstein *et. al.* (1960), for instance, obtained recoveries ranging from 4–62% over a 4½ year period for a variety of soil types. These workers consider the major factors in DDT stability to be organic matter content of the soil and climatic conditions.

For the control of soil insects under pasture persistence of DDT in the soil is a desirable property. At the application rates necessary for the control of grass-grub there is no detrimental effect on subsequent crops. Most of the above work was on cultivated soils with very heavy DDT contamination levels (*ca* 8–800 lb/acre) and is thus not directly applicable to local conditions, in which pp′-DDT at the rate of 2 lb per acre is applied to pasture as a surface dressing. A series of trials was therefore laid down to assess the relative magnitude of DDT breakdown and migration under local conditions of climate, soil type, and application rate. Information obtained from these experiments may help to elucidate the anomalous grub control results obtained in certain limited areas.

The data presented in this paper cover the first 12 months' operation of the initial plot trial at the Otara Research Station, Otahuhu.

EXPERIMENTAL

Details of trial

A replicated plot trial was laid down in June 1960, on existing pasture at the Otara Research Station. (Soil type—Papatoetoe silt loam; pH–5.8). Dry mixed and "wet" mix DDT-superphosphate were applied to damp mown pasture approximately 1 in. in length at rates equivalent to 0, 1, 2, 4, and 8 lb pp′-DDT per acre. The total amount of superphosphate applied in each treatment was kept constant throughout at 8 cwt per acre to eliminate any possible catalytic effects due to the fertiliser background. Three replicates were set out for each treatment, individual plot size being 3.75 ft × 20 ft. Relevant climatic data for the area were available from the Otara meteorological station. The trial area was not stocked. Mowing was performed at weekly intervals, and clippings were allowed to remain on the plots.

Sampling commenced two weeks after top-dressing, when sufficient rain had fallen (2.3 in.) to wash all visible fertiliser

deposits off the foliage. Fifteen soil cores per treatment were taken to a depth of 6 in. and sectioned at 1 in. intervals to determine the vertical distribution of the DDT present in the soil. Plant residues were allowed to remain with the appropriate soil section. Decomposition of DDT was estimated by differences from the observed recovery data. Subsequent samplings were made at three-monthly intervals.

Analytical

Composite soil core material from each depth and treatment was dried at 30°c, and ground in a laboratory mill. Representative samples were extracted with acetone in a Soxhlet apparatus, and suitable aliquots of extract were taken for determination of pp′-DDT by the micro nitration method of Martin and Batt (1958). The original procedure

TABLE 1. DDT CONTENT OF SOIL
(lb. pp′-DDT per inch acre)

Time from application (weeks)	Soil level (in.)	Treatment							
		1 lb pp′-DDT/acre		2 lb pp′-DDT/acre		4 lb pp′-DDT/acre		8 lb pp′-DDT/acre	
		Dry mix	"Wet" mix	Dry mix	"Wet" mix	Dry mix	"Wet" mix	Dry mix	"Wet" mix
2	0—1	0.72	0.87	1.60	1.72	2.95	2.89	7.68	6.35
	1—2	0.03	0.04	0.09	0.12	0.23	0.19	0.57	0.50
	2—3	0.00	0.00	0.07	0.04	0.11	0.07	0.26	0.18
	3—4			0.00	0.00	0.24	0.01	0.10	0.21
	4—5					0.07	0.00	0.00	0.06
	5—6					0.00			0.00
12½	0—1	0.56	0.67	1.35	0.90	2.05	1.83	4.01	3.78
	1—2	0.18	0.20	0.38	0.33	0.71	0.68	1.24	0.92
	2—3	0.16	0.12	0.19	0.18	0.17	0.17	0.31	0.32
	3—4	0.03	0.01	0.09	0.07	0.07	0.10	0.27	0.22
	4—5	0.00	0.00	0.03	0.01	0.01	0.03	0.02	0.03
	5—6			0.00	0.00	0.01	0.02	0.01	0.03
26	0—1	0.44	0.51	1.00	0.81	2.24	1.83	4.04	3.03
	1—2	0.18	0.21	0.43	0.44	0.81	0.66	0.92	0.90
	2—3	0.04	0.10	0.20	0.22	0.32	0.17	0.58	0.49
	3—4	0.01	0.01	0.09	0.04	0.11	0.01	0.11	0.12
	4—5	0.00	0.00	0.02	0.01	0.03	0.02	0.01	0.02
	5—6			0.00	0.00	0.02	0.01	0.06	0.04
39	0—1	0.41	0.36	0.90	0.78	1.42	1.31	3.16	3.29
	1—2	0.11	0.11	0.33	0.34	0.83	0.58	0.98	1.13
	2—3	0.00	0.03	0.17	0.13	0.34	0.12	0.68	0.53
	3—4		0.01	0.11	0.02	0.13	0.01	0.21	0.32
	4—5		0.00	0.03	0.00	0.03	0.00	0.04	0.02
	5—6			0.00		0.00		0.01	0.02
52	0—1	0.21	0.21	0.52	0.31	0.77	0.87	1.95	1.71
	1—2	0.12	0.13	0.42	0.27	0.63	0.60	1.28	1.37
	2—3	0.07	0.04	0.23	0.12	0.31	0.42	0.72	0.61
	3—4	0.03	0.04	0.08	0.06	0.09	0.15	0.20	0.24
	4—5	0.00	0.00	0.00	0.00	0.05	0.07	0.14	0.11
	5—6					0.01	0.00	0.01	0.03

of Martin and Batt was later found to give insufficient purification on certain soil types from other trials. The current method follows the recommendations of the Joint DDT Panel of the *Analyst* (1960), which differs from the original in that clean-up of soil extracts by passage through an activated alumina column, followed by permanganate oxidation, is replaced by treatment in an acid-celite column (Davidow 1950).

All analyses were performed at least in duplicate; and reference specimens of comparable untreated soil, with and without added DDT standard, were run with each set of determinations. Soil density was estimated from sample core dimensions at each depth level, and used to convert analytical data to a lb per acre basis. The method is estimated to be capable of a mean recovery of 95–105%, with a lower limit of detection of the order 0.03 lb pp′–DDT per acre.

RESULTS

Detailed analytical data for the eight treatments throughout the 12-month period at each soil level are presented in Table 1. Results are corrected to DDT-free control as reference in each case. The overall percentage recovery of DDT for treatments is summarised in Table 2.

TABLE 2. DDT RECOVERY
(percentage of pp′–DDT applied)

Time from application (weeks)	Treatment								Mean of all treatments
	1 lb pp′–DDT/acre		2 lb pp′–DDT/acre		4 lb pp′–DDT/acre		8 lb pp′–DDT/acre		
	Dry mix	"Wet" mix	Dry mix	"Wet" mix	Dry mix	"Wet" mix	Dry mix	"Wet" mix	
0									100
2	76	91	88	94	90	79	108	91	90
12½	92	100	102	74	76	71	73	67	82
26	68	83	88	77	89	68	71	58	75
39	52	51	78	64	69	51	63	67	62
52	43	42	63	38	47	53	54	51	49

Analysis of the observed total recovery results for variance indicates no significant difference at the 5% level, either between dry mix and "wet" mix treatments, or between the four rates of application. At each sampling period, the relative distribution of recovered material within the soil levels examined was also virtually identical for each treatment. In view of the lack of significant difference between treatments, overall averages for each sampling period were employed when considering distribution and breakdown trends.

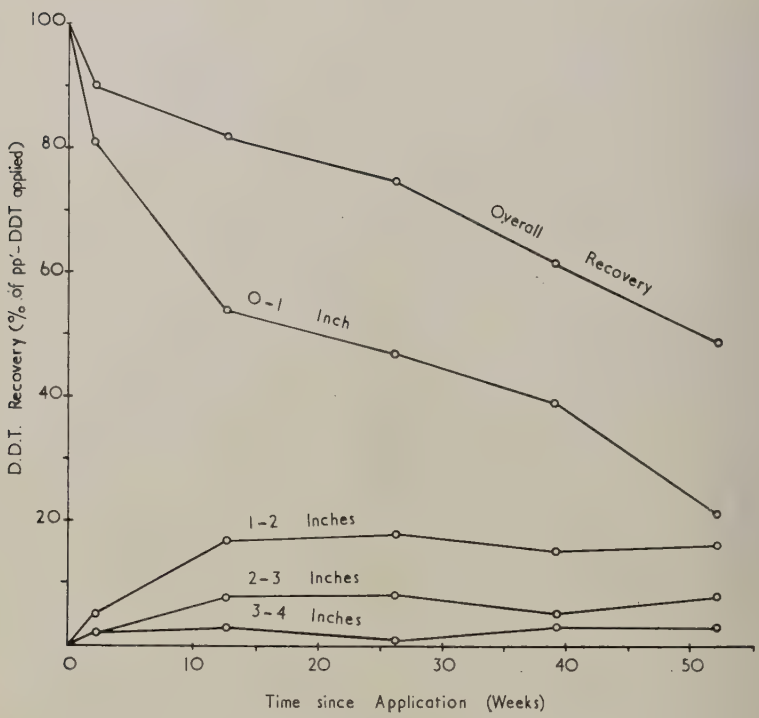

Fig. 1.—DDT recovery in inch soil levels under pasture. (Mean of all treatments).

DISCUSSION

Penetration of DDT into the soil

Recovery of applied DDT at each sampling period from inch increments of soil level is expressed graphically in Fig. 1. From this plot, it would appear that downward migration of DDT under the conditions of the current trial is virtually complete at 3 months from application, possibly owing to attainment of an adsorption equilibrium with the soil. At all subsequent samplings, the distribution of DDT in all soil levels except the top inch remains constant. The decrease in DDT content of the top inch is equivalent to the overall breakdown of the DDT as estimated from reduction in total recovery.

Dry mix vs. "wet" mix DDT-superphosphate. DDT-superphosphate mixtures are prepared commercially either by dry mixing fine DDT powder with mature superphosphate, or by incorporation of DDT during the actual superphosphate manufacturing process ("wet" mixing). The latter method facilitates uniformity of mixing, and

since the DDT is actually recrystallised within a matrix of superphosphate, the product is less likely to suffer segregation during aerial top-dressing operations. On the other hand, the DDT will not become effective in "wet" mix material until the surrounding superphosphate granules are distintegrated by solution of the readily soluble components. In high-rainfall areas, little difference has been observed between the two types of material in time required for initial grass-grub control. Under drier conditions, however, the effect may be more marked, since J. M. Kelsey (pers. comm.) has noted a time differential of 4–6 weeks.

Some difference in penetration between dry and "wet" mix material was therefore expected, at least at the initial sampling period. That none could be detected at two weeks from application would appear to indicate that the rainfall of 2.3 in. during the period (Table 3) was more than adequate for disintegration of the superphosphate granules and liberation of entrained DDT crystals from the "wet" mix material.

TABLE 3. METEOROLOGICAL DATA DURING TRIAL PERIOD

Period (weeks)	Rainfall (in.)	Soil drainage (in.)	Estimated soil temperatures (°F)	
			Mean 0—2 in.	Mean daily max. 0—0.5 in.
0 — 2	2.3	1.8	48	65
2 —12½	9.8	6.2	47	64
12½—26	14.8	3.1	62	81
26 —39	8.8	0.3	68	88
39 —52	16.7	8.8	56	74

Effect of application rate. Although measurable DDT penetration was greatest at higher application rates, as can be seen from Table 1, there was virtually no difference in distribution pattern between any of the treatments. At each sampling, the depth associated with both median and 90% residual DDT levels was determined graphically and found to be independent of application rate within the accuracy of estimation (Table 4).

TABLE 4. DISTRIBUTION WITHIN SOIL OF RESIDUAL DDT

Time from application (weeks)	Median DDT level (in.)				90% DDT level (in.)			
	1 lb rate	2 lb rate	4 lb rate	8 lb rate	1 lb rate	2 lb rate	4 lb rate	8 lb rate
2	0.3	0.3	0.3	0.3	0.5	0.5	0.8	0.6
12½	0.4	0.4	0.4	0.4	1.9	2.0	1.5	1.7
26	0.4	0.5	0.4	0.4	1.6	2.0	1.6	1.8
39	0.3	0.4	0.4	0.4	1.1	1.9	1.7	2.1
52	0.5	0.7	0.7	0.7	2.4	2.3	2.4	2.4

The 90% DDT value was chosen empirically for comparison purposes because of the asymptotic nature of the distribution curves at higher levels.

Mechanism of penetration. Migration of DDT from the soil surface to the zone of maximum grass-grub damage (*ca.* 0 — 3 in.) appears to be primarily due to leaching effects. Earthworm activity may be a minor contributing factor. The aqueous solubility of DDT is of a very low order. Bowman *et al.* (1960) quoted a level of 0.001 p.p.m. However, such true solutions are difficult to obtain, and the authors have been able to prepare stable sols containing up to 1–6 p.p.m. of pp′–DDT which are unaffected by normal laboratory sedimentation and filtration techniques. From the rainfall and drainage data given in Table 3 it is apparent that this solubility effect, possibly in conjunction with some mechanical transfer of ultra-fine particle sizes, is adequate to account for the observed migration of DDT to the lower soil levels. No further penetration has been observed after the initial three months.

Preliminary experiments on other soil types have shown that in some cases adsorption of DDT may be of a much higher order than in this trial, with a possible consequence of greatly reduced penetration. Lack of penetration to an adequate depth may adversely affect the effectiveness of the applied DDT for grass-grub control.

Breakdown of DDT in the soil

The percentage recovery of DDT from the trial plots has been shown to be independent of both type of material and application rate. Mean recovery figures for each sampling period, as given in Table 2, are plotted in Fig. 1, where they may be compared with the corresponding distribution data for each of the upper soil levels. It is clear from this that the whole of the effective breakdown during the first year has taken place within the top inch soil layer.

Decomposition of DDT in the soil may be due to any of a variety of possible factors. Wichmann *et al.* (1946) have shown that light, especially in the ultra-violet region of the spectrum, is capable of inducing breakdown of DDT. Volatility may also cause measurable long-term losses, particularly in the surface layer under high-temperature summer conditions. Catalytic decomposition of DDT in the presence of clay minerals and inorganic salts, notably those of iron, aluminium, and chromium, has been reported by Fowkes *et al.* (1960) and by Fleck and Haller (1945). A number of New Zealand soil types has been examined for catalytic effects, and it has been possible to show that certain soils derived from volcanic ash showers promote decomposition at elevated temperatures. The soil on which this trial is situated is not considered likely to cause unduly high catalytic breakdown. Bacterial decomposition, which is known to induce breakdown of certain organic compounds in the soil, probably plays a major role in the breakdown of the DDT.

Lichtenstein *et al.* (1960) consider the primary factors in DDT decomposition to be soil type and climatic conditions.

Since the percentage recovery of DDT in this trial has been shown to be independent of rate of application, the breakdown mechanism would appear to be unimolecular in type.

TABLE 5. RATE OF DECOMPOSITION OF DDT IN SOIL

Total time (weeks)	Recovery % (a — x)	Rate constant $K = \frac{2.303}{T} \log \frac{a}{a-x}$
0	100	
2	90	0.052
12½	82	0.016
26	75	0.011
39	62	0.012
52	49	0.014
Mean K (3–12 months)		0.013

The rate of decomposition of DDT in the soil during the trial has been calculated (Table 5). After the initial period of rapid decomposition reasonable constancy is attained. Assuming that the minimal residual DDT level for grass-grub control is of the order of 0.5 lb pp′–DDT per acre, it is thus possible to calculate the probable effective life of the rates of application used, under the conditions of this trial (Table 6).

TABLE 6. ESTIMATED EFFECTIVE LIFE OF DDT TOP-DRESSINGS

Rate of application (lb pp′–DDT/acre)	Time to residual of 0.5 lb/acre (years)
1.0	1.0
2.0	2.1
4.0	3.1
8.0	4.1

The breakdown does not appear to be significantly related to soil temperature (Table 3) or peak illumination effects, since decomposition rate does not increase over the summer period (25–35 weeks). Since inorganic catalysis is unlikely in this soil type, the major breakdown factor in this particular case could be bacterial in origin.

Preliminary results from work on other soil types indicate a possibility of accelerated initial breakdown in some cases.

CONCLUSIONS

No significant difference in penetration or breakdown rate could be detected between dry mix and "wet" mix DDT-superphosphates under the conditions of the present trial.

Penetration of DDT into the soil by leaching reached a maximum at three months from application, when an adsorption equilibrium was apparently attained. During the 3–12 month period the amounts present in all soil layers except the top inch have remained constant. The whole of the observed breakdown has occurred within the top inch of soil, possibly owing to bacterial decomposition as a major factor. No significant difference, either in relative distribution within the soil, or in breakdown rate, has been observed between any of the four levels of application, which ranged from 1–8 lb pp′–DDT per acre. The possible effective life of the DDT dressings at these rates for grass-grub control has been estimated to range from one to four years.

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REFERENCES

Allen, N.; Walker, R. L.; Fife, L. C.; Chisholm, R. D.; Koblitsky, L.; Bullock, J. F.; Hodges, C. R.; Hall, E. E. 1954: Persistence of BHC, DDT and toxaphene in soil, and the tolerances of certain crops to their residues. *U.S. Dep. Agric. Tech. Bull. 1090.* 19 pp.

Boswell, V. R.; Clore, W. J.; Pepper, B. B.; Taylor, C. B.; Gilmer, P. M.; Carter, R. M. 1955: Effects of certain insecticides in soil on crop plants. *Ibid. 1121.* 55 pp.

Bowman, M. C.; Acree, F.; Corbett, M. K. 1960: The solubility of C^{14} DDT in water. *J. Agric. Food Chem. 8:* 406–8.

Chisholm, R. D.; Koblitsky, L. K.; Fahey, J. E.; Westlake, W. E. 1950: DDT residues in soil. *J. econ. Ent. 43:* 941–2.

Davidow, B. 1950: Isolation of DDT from fats. *J. Ass. off. agric. Chem. 33:* 130–2.

Dumbleton, L. J.; Kelsey, J. M.; Hoy, J. M. 1948: Chemical control of *Oxycanus cervinatus* Walker. IV. Experiments in 1947 season. *N.Z. J. Sci. Tech. 30A:* 200–5.

Fleck, E. E.; Haller, H. L. 1945: Compatability of DDT with insecticides, fungicides and fertilisers. *J. indus. Engng Chem. 37:* 403–5.

Fowkes, F. M.; Benesi, H. A.; Ryland, L. B.; Sawyer, W. M.; Detling, K. D.; Loeffler, E. S.; Folckemer, F. B.; Johnson, M. R.; Sun, Y. P. 1960: Clay-catalyzed decomposition of insecticides. *J. Agric. Food Chem. 8:* 203–10,

Ginsberg, J. M. 1955: Accumulation of DDT in soils from spray practices. *Ibid. 3:* 322–5.

Ginsberg, J. M.; Reed, J. P. 1954: A survey on DDT—accumulation in soils in relation to different crops. *J. econ. Ent. 47:* 467–74.

Joint DDT Panel—Recommended Methods of Analysis of Pesticide Residues in Foodstuffs, 1960: The determination of small amounts of DDT in flour and other foodstuffs. *Analyst 85:* 600–6.

Kelsey, J. M. 1951: Grass-grub and grass caterpillar control. *N.Z. J. Agric. 83:* 113–22, 195–200.

————1955: Control of grass-grub and subterranean caterpillar. *N.Z. Dep. sci. industr. Res. Ent. Sta. Publ. 14. (2nd revision).* 19 pp.

Lichtenstein, E. P. 1957: DDT accumulation in Mid-western orchard and crop soils treated since 1945. *J. econ. Ent. 50:* 545–7.

Lichtenstein, E. P.; Schulz, K. R. 1959: Persistence of some chlorinated hydrocarbon insecticides as influenced by soil types, rate of application and temperature. *Ibid. 52:* 124–31.

Lichtenstein, E. P.; de Pew, L. J.; Eshbaugh, E. L.; Sleeman, J. P. 1960: Persistence of DDT in some Mid-western soils. *Ibid. 53:* 136–42.

Martin, J. T.; Batt, R. F. 1958: The determination of DDT in plant materials and soil. *Analyst 83:* 340–4.

Wichmann, H. J.; Patterson, W. I.; Clifford, P. A.; Klein, A. K.; Claborn, H. B. 1946: Decomposition and volatility of DDT and some of its derivatives. *J. Ass. off. agric. Chem. 29:* 218–33.

BLOAT IN CATTLE

XXII. Further experiments on treatment and prevention

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ABSTRACT

A number of materials have been tested for their efficacy in the prevention and treatment of bloat in cattle. A water-soluble antifoaming agent was sought which would prevent bloat for a period and be suitable for administration via the drinking water. The most useful found were the neutral, non-ionic "Pluronic" detergents, L61 and L62, which protected cows from bloat for 24 h after 30 ml had been administered. They were, however, too unpalatable to be self-administered via the water supply. The "Pluronics", L61 and L62, were the most effective agents found for the relief of bloated animals.

INTRODUCTION

Following the findings of the South African workers (Quin 1943; Clark 1950; Weiss 1953) with lucerne, it was demonstrated (Johns 1954) that bloat in cattle fed on red clover (*Trifolium pratense* L.) is primarily caused by a foaming of the rumen contents. A number of agents were tried (Reid and Johns 1957) for their usefulness in the prevention and treatment of bloat. Those without antifoaming action were not effective (see also Blake *et al.* 1957).

Dosing with substances selected for their antifoaming properties is now an accepted method of relieving bloated animals. The cheapest and most reliable materials were found to be peanut oil, ruminant tallow, and heavy medicinal paraffin. However, a limitation on their usefulness in bloat prevention is that their duration of effectiveness is only 2–3 h after dosing (Reid and Johns 1957). Consequently, a method of administration was sought whereby the animals had a continuous intake of antifoaming agent while they were grazing on bloat-producing pasture. Reid (1955, 1958) devised the system of applying emulsified oils and tallow directly to the pasture by spraying (see also Johns 1959). This has proved to be a most effective method of preventing bloat where controlled grazing can be practised.

A simpler method of bloat control has been sought. Only partial success has been achieved in attempts to control bloat by adding oils to the drinking water (Reid 1958; Brown *et al.* 1958; Southcott and Hewetson 1958). The rapid passage of oils from the rumen and the irregular drinking habits of cows (Campbell 1958), especially in wet weather, makes this method unreliable, at least with antifoaming agents possessing only a short time of effectiveness. It was considered, however, that if a water-soluble antifoaming agent, effective in controlling bloat at low concentrations, was available, an animal that is drinking infrequently might be protected for sufficient time to permit the material to be administered in the drinking water. This paper presents the results of trials with a variety of agents selected with this end in view, and with materials acclaimed by members of the New Zealand farming community as giving protection from bloat.

EXPERIMENTAL

(1) MATERIALS

A list of materials tested for their bloat-relieving and -preventive properties is given below, together with the respective sources of supply.

1. *Detergents*

   (a) Span 20—Sorbitan monolaureate.
   Span 80—Sorbitan trioleate.
   Atlas Powder Co., U.S.A.

   (b) Polyethylene glycol di-ester of oleic acid.
   Polyethylene glycol mono-ester of oleic acid.
   Ivon Watkins, N.Z.

   (c) "Pluronics". Water-soluble, neutral, non-ionic surface active agents, being straight chain mixed polymers of ethylene and propylene glycols.

   L61 oily liquid, approx. 10–20% ethylene oxide, M.W. approx. 1.800.

   L62: similar to L61 in composition and properties except that its percentage of ethylene oxide varies between 20–30%.

   F68 Flake form, contains 80–90% ethylene oxide.

At a concentration of 0.001% in aqueous solution, L61 and F68 exhibit a surface tension of 45 and 64 dynes/cm respectively. The "Pluronics" are manufactured by the Wyandotte Chemicals Corporation, Michigan, U.S.A. "Pluronic" L62 is distributed in New Zealand as a bloat remedy by Syndet (N.Z.), Ltd under the name of "Bloat-Enz."

2. *Silicones*

(a) Rumoxane—a proprietary bloat remedy containing polymethyl silicone.
Willows Francis Ltd, United Kingdom.

(b) SAG 470—a silicone antifoam emulsion for industrial use manufactured by the Union Carbide Company, U.S.A.

(c) Dymasyl gastric defoamer, containing 28.2% dimethylpolysiloxane and 1–3% silicon aerogel, and supplied by Swift and Co., N.Z. Manufactured by Dow Corning Corporation, U.S.A.

3. *Other antifoaming agents*

(a) Blotrol—a proprietary bloat remedy containing 2 Acetoxy-1, 2, 3 propane-tricarboxylic acid tributyl ester. Manufactured by Chas. Pfizer and Co., U.S.A., and supplied by Sattherthwaite and Co. (N.Z.), Ltd, Christchurch, N.Z.

(b) Citroflex-A-8 Acetyl tri-2-ethylhexylcitrate.
Chas. Pfizer and Co., U.S.A.

4. *Antibiotics*

(a) Hibitane Bis [p-chlorophenyldiquamido-] hexane.
Imperial Chemical Industries (N.Z.), Ltd.

(b) Streptomycin.
Glaxo (N.Z.), Ltd.

(c) Neomycin sulphate.
Glaxo (N.Z.), Ltd.

5. *Other remedies*

(a) Slaked lime prepared and supplied by Mr C. J. Aplin, Tirau.

(b) Cobalt "bullets", "Permaco" brand, active ingredient 90% cobalt oxide, Nicholas Pty., Ltd, Victoria, Australia.

(c) Rawleigh's "Colic Ease". A proprietary remedy for treatment of horse colic (not bloat) manufactured by W. T. Rawleigh and Co., Ltd., Wellington, N.Z.

(d) Ethyl and n-Butyl alcohols (equal mixture).

(2) METHODS

Details of the feed, the feeding regime, and the grading system for assessing bloat were given in Part 1 of this series (Johns 1954). Bloat-susceptible, non-bloating, identical-twin cows were used.

Two methods of screening compounds for effectiveness in bloat control were employed (Reid and Johns 1957).

(a) *Prevention of bloat.* It is easier to prevent the formation of a foam of the high viscosity type experienced in bloated animals than to destroy it once it is formed. It has been found by experience that if an agent is of no use as a prophylactic it is useless therapeutically. However, it was not always possible to have a continuous run of bloating so that prefeeding of the agents could be systematically carried out. Hence, some agents were screened by method (b) whenever bloated animals were available.

(b) *Treatment of bloat.* The effectiveness of a material in relieving a bloated animal was determined from the deflation time after dosing. This was taken as the interval between dosing and the complete disappearance of all traces of bloat as detected by palpation of the left flank. An experiment was not considered to give a positive result unless the untreated control twin remained bloated. From previous experience it was known that effective agents such as the various vegetable and mineral oils have a mean deflation time of approximately 32 min (Reid and Johns 1957).

Where an agent was effective prophylactically, it was tested to see for how long it would prevent bloat by observing the behaviour of the animal at subsequent feeds for up to two days.

Unless otherwise stated, the introduction of the material into the animal was carried out by diluting with water to approximately 400 ml, shaking vigorously in a large-necked bottle, and dosing over the back of the tongue.

In the case of agents tested in drinking water, the animals were separated into two groups, one of which had access only to treated, and the other only to untreated, drinking water.

RESULTS

1. *Detergents*

(a) Span 20 and 80 were used prophylactically in dose rates up to 50 ml given before feeding the cows on bloat-producing clover. Bloat was prevented during this first feed but no "carry-over" protection resulted for subsequent feeds. At the 50 ml level both preparations relieved bloated animals in deflation times which ranged from 13 to 46 min.

(b) Trials with the mono-and di-esters of oleic acid showed that animals dosed with 30 ml of either of these two materials bloated to Grade III during the feed following drenching. A bloated animal treated with the mono-ester did not deflate any more rapidly than its untreated control twin and bloated severely again when fed 3 h later. A similar result was achieved with 60 ml of the di-ester.

(c) Extensive trials were carried out with the "Pluronics" L61 and L62. In stall-fed, non-lactating twins, 15 ml controlled bloat for a 24 h-period when used either before the morning or afternoon feeding period. In lactating cows under normal grazing

conditions, it was found that the minimum amount needed for complete protection from bloat for 24 h was 30 ml.

Complete control of bloat was achieved in a commercial herd by using a mixture of one part "Pluronic" L62 and two parts of water and administering this with a drenching-gun connected to a knapsack reservior (N. J. Phillips Pty., Ltd, Sydney). Large animals were dosed with 120 ml of the mixture and small animals 60 ml (i.e., 40 ml and 20 ml of L62).

"Pluronic" L62 was added to drinking troughs (30 ml in 20 gal) in the only source of water available to a dairy herd. The treated water was not consumed by the herd even after several days, whereas animals in the control group drank normally. The extremely bitter taste of the L62, though diluted, presumably was responsible for the lack of drinking by the group with the treated water.

The two "Pluronics" were used on numerous occasions for treating bloated animals and appeared to be very similar in their speed of action. The mean deflation times for drenching with 30 ml of L62 was 25.4 $\pm$ 4.0 min.

2. *Silicones*

(a) Rumoxane. This proprietary bloat remedy was found to be quite ineffective in the treatment and prevention of bloat. Bloated cows drenched with 100 ml or 200 ml of Rumoxane failed to respond in all cases. A free-grazing animal bloated to Grade III 1 h after the administration of 100 ml of the material, while another bloated severely after 50 ml. The recommended dose is 100 ml.

(b) The preparation "Sag 470" failed to prevent bloat when administered at the 30- or 60 ml-level before feeding. The administration of 30 ml to a moderately bloated animal failed to deflate it in 65 min.

(c) Only a small quantity of the "Dymasyl Gastric Defoamer" was available for test, Treatment with 8 ml failed to deflate a bloated animal any faster than its control twin, and bloat was experienced during the next feeding period.

3. *Other antifoaming agents*

(a) When administered prior to the morning feed, 30 ml of "Blotrol" were sufficient to prevent bloat in the test animals for both the morning and afternoon feeding periods. Administration of 30 ml prior to the afternoon feed did not prevent bloat the following morning.

Based on the treatment of 12 cases of Grade III bloat or higher, the mean deflation time was 37.2 $\pm$ 3.4 min. This is a very similar result to that obtained for peanut oil in these trials.

(b) The administration of 30 ml of "Citroflex-A-8" before the morning feeding period prevented bloat for that feed but the

animals bloated during the afternoon. When it was used to relieve bloating animals, the deflation times varied between 20 and 75 min.

4. *Antibiotics*

(a) The wide spectrum antibiotic "Hibitane" was administered prophylactically in the evening in doses ranging from 1 to 4 g. In all cases bloat was experienced the day after treatment. In the case where 4 g was given, the animal bloated more severely than any of the other test animals.

(b) When streptomycin or neomycin were given in doses of 500 mg in the evening the animals bloated the next day.

5. *Other remedies*

(a) It has been claimed by many farmers situated in the central areas of the North Island of New Zealand that slaked lime, treated by a particular procedure and added to water troughs, protects cows from bloat. Lime was added to water troughs for three weeks, at the recommended concentration. Sixty-six cases of bloat were recorded in the experimental group of seven cows, of which 15 cases were Grade III or higher. Dosing animals with lime over a range 5–30 g produced, if anything, more bloat in the treated than in the untreated cows.

(b) In the Wairarapa area of New Zealand it was claimed that cobalt administered in the form of "bullets" would protect cows from bloat.

Two cows—one member of each of two sets of identical twins—were given cobalt bullets. The bloat incidence in the week following administration was very similar in both the treated and the control member of the twin set. The bullets were left in the animals for three months, and at the end of that time there was no reduction of the frequency or severity of bloat in the treated animal. Dosing animals with cobalt acetate to give a rumen concentration of cobalt of 0.2–1.0 p.p.m. was without beneficial effect on bloat.

(c) Rawleigh's "colic-ease" has been used quite widely by dairy farmers for the relief of bloated animals. Its usefulness was investigated following complaints of taint in the Palmerston North milk supply and in the Glaxo Laboratories' factory supply. It was found that "colic-ease" was not as effective as the mineral or vegetable oils in deflating bloated animals and that there was no carry-over of bloat control past the feed following treatment.

Investigations carried out by the Dairy Research Institute showed that drenching with "colic-ease" resulted in a strong, foreign flavour in the milk obtained from the first milking following drenching.

(d) On the basis of laboratory tests with artificial foams and rumen contents, Tolgyesi (1959) suggested that a 30 ml mixture of equal amounts of ethyl and n-butyl alcohol should be used for the

treatment of bloat. Using twice this quantity, it was found that the mixture neither prevented bloat when administered before feeding nor caused any decrease in the deflation time of a bloated animal.

DISCUSSION

The results obtained here confirm earlier results of Reid and Johns (1957) in that, except in the case of the antibiotic penicillin (Barrentine *et al.* 1956), agents without antifoaming action are of little use in the prevention or treatment of bloat. The results also extend the range of silicones tried and confirm that, although these compounds are well known for their antifoaming properties, those so far tried are quite ineffective in bloat control.

Besides the dose rates given in the Results section, most agents were tested over a wide range of dose levels to determine the safe minimum level that could be relied on for an effective agent.

Some detergents are active as antifoaming agents for the high viscosity foams of the protein-saponin type (Reid and Johns 1957). However, it still appears to be a matter of trial and error to determine which is likely to be the most effective agent in both speed of relief and length of time the animal will be protected from bloat No laboratory method of testing has yet been devised which can be used successfully to screen possible agents (e.g., Tolgyesi, with ethyl and n-butyl alcohol quoted above).

The neutral, non-ionic surface active agents of the "Pluronic" type with considerable water solubility appeared to offer the greatest possibility of meeting the requirements of a useful agent. From the trials it seems that L61 and L62, which have the ability to cause the greatest depression of the surface tension of water at low concentrations, are the most useful. They are the most effective agents so far tried for the relief of bloated animals and have the added advantage of protecting these animals from bloat for a further 24 h when used at the 30 ml level. Unfortunately, owing to their bitter taste, they are not suitable for administration via the drinking water.

Oxford (1959), in laboratory tests, found that above a concentration of 0.05%, L62 is lethal to *Epidinium*, which is one of the major oligotrich ciliate protozoa present in the clover-fed twins. However, at the dose level used (30 ml), no harmful effects were noted on either the protozoan population (R. T. Clarke pers. comm.) or on milk yield and composition (Dairy Research Institute, unpublished results).

Other surface active agents such as Span 20 and 85, and the proprietary antifoaming agents "Blotrol" and "Citroflex" A-8 are effective as short-term antifoaming agents but do not give the long-term protection afforded by the "Pluronics."

The various New Zealand farmers' remedies, some of which were in widespread use, did not stand up to the test of controlled experiments. The use of cobalt, however, appeared to have a possible scientific basis, as Thorn and Helm (1957) discovered that

cobalt at a concentration of 0.2–1.0 p.p.m. almost entirely abolished the tendency for beer to overfoam. The hypothesis was proposed "that cobalt, by complexing with certain nitrogenous constituents of the beer, produces surface-active substances which inactivate the nuclei responsible for overfoaming." Whatever its mechanism of action, cobalt is of no use in preventing bloat in cattle when used at this concentration.

It is doubtful whether it is possible to find a surface-active agent that can be administered in the drinking water and give the complete protection realised by pasture spraying of oils or tallow (Johns 1959). On account of the wide variations in the drinking habits of individual cows in a herd it would have to be active in very low concentrations to protect all animals. Campbell (1958) found that herd average consumption varied from 2 to 10.5 gal per cow per day. On one light-drinking day over 25% of the cows did not drink at all, 40% drank between 2 and 4 gal, while the biggest drinker consumed 7 gal.

Even though the method has these limitations a palatable antifoaming agent with the other properties of L62 would probably reduce the incidence and severity of bloat in hilly areas where the terrain does not permit a tractor to be used for pasture spraying.

Another obvious method of administration of antifoaming agent would be in a concentrate fed at milking time. However, under New Zealand conditions a considerable proportion of the average dairy herd will not consume any concentrates when offered during the bloat season.

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REFERENCES

Barrentine, B. F.; Shawver, C. B.; Williams, L. W. 1956: *J. Anim. Sci. 15:* 440–6.
Blake, J. T.; Jacobson, N. L.; Allen, R. S. 1957: *Amer. J. vet. Res. 38:* 756–60.
Brown, L. R.; Johnson, R. H.; Jacobson, N. L.; Homeyer, P. G. 1958: *J. Anim. Sci. 17:* 374–85.
Campbell, I. L. 1958: *Dairy-fmg. Annu.:* 53–60.
Clark, R. 1950: *J. S. Afr. vet. med. Ass. 21:* 173–8.
Johns, A. T. 1954: *N.Z. J. Sci. Tech. A.36:* 289–320.
————1959: *N.Z. J. Agric. 99:* 2–9.
Oxford, A. E. 1959: *N.Z. J. agric. Res. 2:* 365–74.
Quin, J. I. 1943: *Onderstepoort J. vet. Sci. 18:* 113–7.
Reid, C. S. W. 1955: *Proc. 17th Conf. N.Z. Grassl. Ass.:* 70–9.
————1958: *Agric. Rev., Lond. 4:* 36–43.
Reid, C. S. W.; Johns, A. T. 1957: *N.Z. J. Sci. Tech. A.38:* 908–27.
Southcott, W. H.; Hewetson, R. W. 1958: *Aust. vet. J. 34:* 136–9.
Thorn, R. S. W.; Helm, E. 1957: *J. Inst. Brew. 63:* 415–35.
Tolgyesi, G. 1959: *Mag. allator Lapja 18:* 148–51. (Seen only in abstract in *Vet. Bull. 29:* 140 abstr. 794.)
Weiss, K. E. 1953: *Onderstepoort J. vet. Res. 26:* 241–50.

ASPECTS OF THE FAECAL EXCRETION OF SELENIUM BY SHEEP

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ABSTRACT

When 12 μg ^{75}Se-selenious acid was introduced into the rumens of sheep 51% was recovered in the dung over a 72-hour period. This confirms that the major route of excretion of selenium in ruminants is not via the urine as in monogastric animals. Chemical fractionation of the dung showed that most of the selenium was present in forms insoluble in water or solvents. The insoluble fraction appeared to be approximately half inorganic and half protein-bound. In a pot experiment negligible amounts of selenium were taken up from dung into herbage of perennial ryegrass over a period of 53 days.

INTRODUCTION

It has been found in recent years that trace amounts of selenium (0.01 p.p.m.) have important nutritional and metabolic functions in many animal species (Schwarz and Foltz 1957; Patterson *et al.* 1957; Sharman 1960; Tagwerker 1960; Schultze 1960). In New Zealand Drake *et al.* (1960) reported that oral administration of small quantities of selenium either as selenite or selenate prevented "white muscle disease" in sheep and improved the rate of growth of weaned lambs.

In monogastric animals it has been found that the chief route for the excretion of selenium is in the urine (Smith *et al.* 1938). Cousins and Cairney (1961) reported that oral dosing of sheep with 5 mg sodium selenite led to a high faecal excretion of selenium, even on low levels of feed intake. The bulk of the faecal selenium was found to be in a water-insoluble form. As the amount of selenium used by these authors far exceeds the normal daily intake of selenium by grazing sheep, it was desirable to repeat the experiments using lower selenium levels in case the high faecal excretion was due to the high level of selenium used.

An experiment was therefore carried out on the faecal excretion of ^{75}Se-selenite introduced into the rumens of sheep. The use of ^{75}Se of high specific activity enabled a chemical fractionation of the ^{75}Se-labelled compounds present in the dung to be made. The availability of the ^{75}Se in the dung to perennial ryegrass plants was also determined in a pot experiment.

EXPERIMENTAL

Faecal excretion experiment

Selenium-75 was obtained from Oak Ridge National Laboratory, U.S.A., as selenious acid in 1.3 N HCl, specific activity 7,900 mc/g Se.

Two healthy wethers, one aged 2½ years (105 lb), the other of indeterminate age (140 lb), were used. The radioactive selenium was introduced into the rumen by piercing the rumen wall with a trocar and cannula and injecting 85 μc ^{75}Se-selenious acid (12μg Se) contained in 20 ml water through plastic tubing into the rumen. Care was taken to avoid contact of the radioactive solution with metal (Peterson and Butler in press). After injection, the animals were allowed to graze on ryegrass/white clover pasture growing on Manawatu silt loam. The pasture had not been grazed for a considerable period and was free of sheep dung, which could easily be collected from it without soil contamination.

All the dung dropped was collected at 8 a.m. and 4 p.m. for 3 days, the first collection being made 16 hours after ^{75}Se-selenite administration. A collection of dung excreted over an 8-hour period was made at 4 p.m. on the 8th day. No rain fell over the dung collection periods, so that leaching of the dung did not occur.

All dung samples were freeze-dried and powdered. Suitable sub-samples were then counted with constant geometry, using a scintillation probe (Phillips PW 4111) coupled to the necessary Phillips scaling gear. Standard solutions of ^{75}Se-selenious acid were also counted with the same geometry; this was achieved by counting the solution in the same container and the same volume as for the dung samples. Carrier sodium selenite was added ($10^{-3}M$) to prevent adsorption effects. Since ^{75}Se emits only gamma-radiation of relatively high energy no self-absorption corrections were required. Corrections were made for background and for radioisotope decay.

Chemical fractionation of the ^{75}Se-labelled dung

The sample excreted over the period 24–40 hours after ^{75}Se-selenite administration (which contained the greatest amount of ^{75}Se) was subjected to a detailed chemical examination. A suitable aliquot (35 g) of the freeze-dried powdered material was extracted with the following sequence of solvents:—

(1) Ether, for 8 hours in a Soxhlet apparatus.

(2) Chloroform, for 8 hours in a Soxhlet apparatus.

(3) Aqueous ethanol (80% v/v). The material was boiled for 10 min. with two 250 ml portions and washed with a third 250 ml portion.

(4) Water. Three extractions were made with 250 ml portions of boiling water for 10 min.

The extracts were clarified by filtration or centrifugation, and concentrated using a rotary film evaporator at a bath temperature of 40°c. Suitable aliquots were assayed for ^{75}Se-activity.

The material remaining after solvent extraction was freeze-dried, and 1 g quantities were shaken at 37°c. with 15 ml volumes of the following reagents and the radioactivity brought into solution determined:—

(1) *Tris*—hydroxymethylaminomethane buffer ("Tris"), pH 7.6, 0.01 *M*, containing 50 μg/ml chloramphenicol for 40 hours.

(2) Sodium phosphate buffer, pH 7.6, 0.01 *M*, containing 50 μg/ml chloramphenicol for 40 hours.

(3) Sodium ethylenediaminetetra-acetate, "Versene", pH 7.6, 0.01 *M*, containing 50 μg/ml chloramphenicol for 40 hours.

(4) Cupferron, 0.01 *M*, in 0.01 *M* phosphate buffer, pH 7.6, 40 hours.

(5) *o*-Phenanthroline, 0.01 *M*, in 0.01 *M* phosphate buffer, pH 7.6, 40 hours.

(6) Diethyldithiocarbamate, 0.01 *M*, in 0.01 *M* phosphate buffer, pH 7.6, 40 hours.

(7) Water saturated with H_2S, 64 hours.

(8) Carbon disulphide, 64 hours.

(9) Saturated bromine-water, 16 hours.

(10) Ammonium polysulphide solution, 6 *N* (Vogel 1945), 16 hours.

Treatments 1 to 6 were tests of the possibility that ^{75}Se was chelated with heavy metals. Treatments 7 and 8 were tests for elemental ^{75}Se (red allotrope), and treatments 9 and 10 were tests for insoluble inorganic ^{75}Se.

The action of proteolytic enzymes on the faecal material after solvent extraction was also examined. A suitable aliquot (500 mg) was shaken with 10 ml phosphate buffer (0.01 *M*, pH 7.6), containing 100 mg chymotrypsin and 0.5 mg chloramphenicol, for 40 hours at 37°c. The suspension was filtered off, washed with water, and incubated with 100 mg trypsin and 0.5 mg chloramphenicol in 10 ml phosphate buffer (0.01 *M*, pH 7.6), for 40 hours at 37°c. The suspension was again filtered off, washed with water, and shaken with 10 ml bromine-water for 20 min. at room temperature. All filtrates were concentrated under reduced pressure and ^{75}Se-activity determined. The radioactivity retained on filters and in the residual material was also determined, so that a balance sheet of the recovery of radioactivity could be constructed. The enzyme preparations used were trypsin (crystallised, substantially salt free) and α-chymotrypsin (crystallised, salt-free), from Sigma Chemical Company, St. Louis, U.S.A.

The trypsin and chymotrypsin digests were combined and dialysed against water. The diffusate was freeze-dried, and the residue dissolved in a small volume of water and applied to Whatman No. 3 MM paper as a band 2.8 cm wide. Paper chromatography in butanol-pyridine-water (1 : 1 : 1 v/v) was carried out and the distribution of ^{75}Se-radioactivity determined using the rate-meter described elsewhere (Peterson and Butler in press).

Availability to ryegrass plants of ^{75}Se from dung

The sample of dung excreted by the sheep over the period 16–24 hours after ^{75}Se-selenite administration was used for this experiment. Finely-powdered dung was mixed with sand in plastic pots at the rate of 10 g, 20 g, and 30 g of dung per 350 g sand, with 2 pots for each treatment. Seeds of New Zealand certified perennial ryegrass were sown in the pots and allowed to germinate and grow in a heated glasshouse at 18°c. Care was taken not to water to the point of drainage to avoid leaching of ^{75}Se. Hoagland-Arnon (1938) nutrient solution was applied at 14-day intervals.

On the 30th and 53rd day after sowing the plants were defoliated to a height of 2 cm and the herbage freeze-dried. On the 53rd day the roots and media were also freeze-dried. All samples were finely cut with scissors, and suitable aliquots were counted using a scintillation probe fitted with a well-type crystal (Phillips PW 4118).

RESULTS

Faecal excretion experiment

In Table 1, the ^{75}Se-radioactivity observed in the various dung samples is given on a total and unit dry weight basis. It will be seen that faecal excretion of ^{75}Se was maximal about 24 hours after administration. The amount of radioactivity recovered in the dung of the 2 animals over the period 0–72 hours was 51% of the amount of ^{75}Se-selenite administered.

TABLE 1. ^{75}SE-RADIOACTIVITY OBSERVED IN SHEEP DUNG AFTER INTRODUCTION OF 12μg ^{75}SE (85 μc*) AS SELENIOUS ACID INTO THE RUMEN

Sample	^{75}Se activity	
	Total (counts/min $\times$ 10^{-3})	Per g dry wt. (counts/min)
0 — 16 h	523	1,915
16 — 24 h	563	3,180
24 — 40 h	1,195	3,140
40 — 48 h	517	1,501
48 — 64 h	530	1,147
64 — 72 h	137	500
160 — 168 h	8.3	53

*Equivalent to 6.79 $\times$ 10^8 counts/min. with the scintillation detector used.

Chemical fractionation of ^{75}Se-labelled dung

Ether, chloroform, and 80% (v/v) aqueous ethanol successively extracted 1.9%, 0.7%, and 5.0% respectively of the total radioactivity of the 35 g sample of dung. A larger percentage, 13.8%, was extracted by water; this was probably an over-estimate of the water-soluble fraction, because of the difficulty of clarifying this extract from colloidal material.

Less than 5% of the total radioactivity in each of the 1 g samples was extracted by phosphate buffer (pH 7.6), "Tris" buffer (pH 7.6), versene (pH 7.6), cupferron, o-phenanthroline, diethyldithiocarbamate, saturated hydrogen sulphide solution, or carbon disulphide. There was thus no evidence for the chelation of ^{75}Se with metals or for the presence of ^{75}Se as the red selenium allotrope.

Treatments with both ammonium polysulphide solution and bromine water resulted in extraction of large portions of the total radioactivity, the figures being 61% and 66% respectively. This indicates the presence of ^{75}Se in insoluble inorganic form.

TABLE 2. PERCENTAGE OF ^{75}SE-RADIOACTIVITY EXTRACTED BY PROTEOLYTIC ENZYMES AND BY BROMINE-WATER FROM DUNG WHICH HAD BEEN SOLVENT EXTRACTED

Treatment	^{75}Se extracted	
	Counts/min.	%
Chymotrypsin	1,118	37.6
Trypsin	284	9.6
Bromine-water	939	33.4
Residues	499	16.8
Filters	132	4.4

In Table 2 the percentage of radioactivity extracted by successive treatments of solvent-extracted dung with chymotrypsin, trypsin, and bromine-water are presented. It will be seen that the proteolytic enzymes extracted a substantial proportion of the total radioactivity. The ratemeter tracing of the chromatogram prepared from these digests showed 2 broad peaks with maximum at R_F 0.08 and 0.30 respectively. Although the activity was too low to permit attempts at identification by the use of a number of chromatographic and electrophoretic systems, it would appear that these peaks were seleno-aminoacids or seleno-peptides, since they were released from the extracted dung residues by the proteolytic enzymes.

Availability to ryegrass plants of ^{75}Se from dung

In Table 3, a balance sheet is shown for the distribution of ^{75}Se in herbage at the first and second cuttings and in roots and media at the second cutting. The percentage recovery of ^{75}Se is also

shown. It will be seen that very small proportions of the total radioactivity in the dung were taken up into the herbage. Samples of roots dissected from the media at the second cutting also contained little radioactivity. The root systems had grown through the sand-dung mixture particularly well at the lower level of dung added; at the highest level root growth was somewhat inhibited.

TABLE 3. UPTAKE OF ^{75}SE FROM DUNG BY PERENNIAL RYEGRASS PLANTS

(All figures are given as % of the total radioactivity added initially. The first and second cuttings were made 30 and 53 days after sowing respectively. Data are given for both replicates of each treatment.)

Amount dung per pot (g)	First cutting	Second cutting		Total recovery
	Herbage	Herbage	Roots plus medium	
10	0.10	0.22	87.1	87.4
	0.43	0.21	67.8	68.4
20	0.08	0.16	87.9	88.2
	0.07	0.10	90.1	90.3
30	0.02	0.10	86.9	87.0
	0.11	0.07	86.6	86.8

DISCUSSION

Cousins and Cairney (1961) reported the recovery in dung excreted over a period of 72 hours of 40% of 5 mg selenium given as single oral doses of sodium selenite to each of 3 hoggets. In the experiment reported here a similarly high faecal excretion of selenium was noted when ^{75}Se-selenious acid was introduced into the rumen at the more physiological level of 12 μg Se. It would therefore appear that, in contrast with the experiments with monogastric animals, there is a high faecal excretion of selenium given as selenite over a wide dose range.

The daily intake of selenium by sheep grazing New Zealand pastures can be calculated from the analyses of Watkinson (1960) to be of the order of 60μg. From the experiments of Peterson and Butler (in press) on the uptake and assimilation of ^{75}Se-selenite by pasture plants it appears probable that the selenium in pasture herbage is present predominantly as seleno-aminoacids either in the free state or bound to proteins. It would therefore seem to be of importance to establish whether the faecal excretion by sheep of selenium ingested in these forms is as great as in the case of selenite. At relatively high selenium levels retention in the tissues of ruminants has been observed to be much greater when "naturally occurring organic selenium" is ingested than when selenite is ingested (Sharman 1960).

The observation of Cousins and Cairney (1961) that most of the faecal selenium is present in insoluble form was confirmed. From the amounts of radioactivity extracted by successive treatments of solvent-extracted dung with trypsin, chymotrypsin, and bromine-water it can be concluded that approximately half the insoluble material was protein-bound and the other half was inorganic. The nature of the insoluble inorganic material cannot be definitely stated; the various possibilities are elemental selenium, or selenite or selenide in heavy metal combination. The inability of various chelating agents to extract ^{75}Se from solvent-extracted dung is probably evidence against the presence of heavy metal selenites. The insolubility of ^{75}Se from dung in carbon disulphide is not evidence against the presence of elemental selenium, since only the red allotrope shows solubility in this solvent. Although the red allotrope would be formed first, transition to the grey allotrope would occur with storage (Friend 1931).

The availability to pasture plants of the selenium excreted by sheep is of importance. Because of the dosing of sheep with sodium selenite in New Zealand the possibility that herbage growing in areas of high fertility (such as stock camps) could absorb large amounts of faecal selenium has to be considered. Under the conditions of the pot experiment described here perennial ryegrass took up very small quantities of selenium from dung. No attempt was made to simulate field conditions in this experiment, but since the dung was in a finely-divided form throughout the pots it is considered that the opportunity for selenium to be absorbed was more favourable than would occur in the field. It should be remembered that differences in the manner of microbiological breakdown of the dung under different environmental conditions could strongly affect the availability of faecal selenium to plants. Also other plant species may be able to absorb selenium from dung to a greater extent.

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REFERENCES

Cousins, F. B.; Cairney, I. M. 1961: *Aust. J. agric. Res. 12:* 927.

Drake, C.; Grant, A. B.; Hartley, W. J. 1960: *N.Z. vet. J. 8:* 4.

Friend, J. Newton 1931: "Textbook of inorganic chemistry." Vol. 7, part 2. p. 291. Griffin, London. 420 pp.

Hoagland, D. R.; Arnon, D. I. 1938: *Calif. Agric. Exp. Sta. Circular 347:* 39 pp.

Patterson, E. L.; Milstrey, R.; Stokstad, E. L. R. 1957: *Proc. Soc. exp. Biol., N.Y. 95:* 617.

Peterson, P. J.; Butler, G. W. (in press): *Aust. J. Biol. Sci. 15:*

Schultze, M. O. 1960: *Annu. Rev. Biochem. 29:* 391.

Schwarz, K.; Foltz, C. M. 1957: *J. Amer. chem. Soc. 79:* 3292.

SHARMAN, G. A. M. 1960: *Proc. Nutr. Soc. 19:* 169.

SMITH, M. I.; WESTFALL, B. D.; STOHLMAN, E. F. 1938: *Publ. Hlth Rep., Wash. 53:* 1199

TAGWERKER, F. J. 1960: *Agric. vet. Chem. 1:* 23, 78.

VOGEL, A. I. 1945: "Textbook of qualitative chemical analysis." 3rd ed. p. 542. Longmans, Green, London. 578 pp.

WATKINSON, J. H. 1960: *Analyt. Chem. 32:* 981.

Pithomyces chartarum SPORES ON PASTURE AND THEIR RELATION TO FACIAL ECZEMA IN SHEEP

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ABSTRACT

Thornton and Sinclair's method of counting spores from washed herbage samples and Brook's volumetric spore trap were used to measure the seasonal fluctuation in numbers of spores from *Pithomyces chartarum* on pasture, and the results obtained from the two methods were found to agree closely.

A strong positive correlation was obtained between estimated numbers of spores on pastures and the degree of facial eczema in lambs grazing thereon.

Strong negative correlations were obtained between spore numbers and liveweight gains of the lambs, and between degree of liver damage and liveweight gains.

INTRODUCTION

In 1958 *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis (syn. *Sporidesmium bakeri* Syd.) was shown to cause facial eczema disease in sheep (Thornton and Percival 1959; Percival 1959). A major aim of subsequent field investigations has been to evolve methods for measuring the abundance of the fungus on pastures and to determine levels at which it causes the disease in animals grazing thereon.

Thornton and Sinclair (1960) devised a technique which involved washing freshly cut pasture samples and counting the spores recovered. No attempt was made to estimate the total amount of *P. chartarum*, including both spores and mycelium. The method estimated numbers of spores only, and in trials at Manutuke it was shown that the accuracy and reproducibility of the technique was good, and that high spore numbers were associated with severe facial eczema. Counts of 4×10^5 spores per g, dry weight of pasture, and above, produced serious liver damage.

Brook (1959) described a mobile volumetric spore trap which measured the number of spores per cu. ft. of air above pasture beaten mechanically by the passage of the trap. In one of a series of trials in the Waikato district the mean numbers of spores trapped by this machine were strongly correlated with the degree of facial

eczema liver damage in lambs grazing the trial area (P. J. Brook and F. T. Lees pers comm.).

However, these two methods of estimating spore numbers were not first applied on an experimental scale, either at Manutuke or in the Waikato, until active growth of *P. chartarum* had already taken place; and a complete record of the seasonal growth of the fungus and the levels at which facial eczema first occurred was not obtained. A more thorough evaluation of these methods was therefore essential before they could be used to predict the occurrence of facial eczema in the field.

This paper describes further experiments carried out at the Manutuke Research Station during the summer and autumn of 1960.

Spore numbers estimated by both methods were compared with each other and with the severity of facial eczema liver damage in grazing lambs. Correlations between spore numbers and liveweight gains, and between degree of liver damage and liveweight gains, were also investigated. One trial was planned specifically for these purposes. Others, although primarily designed to measure the effect of various fungicidal and managerial treatments on the abundance of *P. chartarum*, provided an opportunity to correlate spore numbers with liver damage and liveweight gains regardless of treatments which are not further considered here.

MATERIALS AND METHODS

Trial 1

A comparison of spore numbers estimated by the two methods, in relation to facial eczema liver damage, was carried out on two adjacent acre fields which were given prior treatments planned to produce widely different spore populations. The fields and treatments were as follows:—

Field 10 was set-stocked at a rate somewhat less than full utilisation, to provide a typical rough sheep-grazed pasture.

Field 37 was not grazed after mid-October. It was mown at intervals and clippings spread to provide a suitable medium for *P. chartarum*.

Washed grass counts were done on both fields from 1 December 1959 and spore trap counts concurrently from 5 January 1960 when a machine became available. When spores first appeared in appreciable numbers both fields were set-stocked with weaner lambs for six successive 3 week periods from 23 December 1959 to 3 May 1960. At the end of each period lambs were slaughtered and their livers were graded for facial eczema damage.

Trial 2

Weekly spore trap counts were made on nine uniform 1 acre fields, each grazed by 10 lambs in three successive 4–5 week periods between 16 January and 26 April 1960.

Liver damage and liveweight gains were recorded for all periods, which were arranged, as far as possible, to coincide with marked changes in spore numbers. The fields involved were managed as uniformly as possible. For several months before the trial they were stocked at similar rates, and from 11 December 1959 they were all mown at regular intervals to maintain the pasture at a height of 3 in. and to encourage growth of *P. chartarum* by a continuous supply of fresh litter as on Field 37 in Trial 1.

Trial 3

Four adjacent 2 acre fields—numbers 1 to 4—were subjected to different grazing management from 1 October 1959 to mid-January 1960 in order to create extremes in the amount of pasture litter accumulating during spring and summer before the beginning of the facial eczema season. By 26 January this had been achieved, and the fields were then set-stocked with 20 lambs each, which grazed continuously until they were slaughtered on 26 May. Spore trap counts were made each week concurrently with those in Trial 2, and liver damage was graded at the end of the experiment.

In addition to the trials at Manutuke, fields on two farms within 6 miles of the Station were examined by spore trap each week. By arrangement with the owners these fields were grazed continuously by small groups of lambs which were slaughtered at the end of the season. The results obtained from these farms have consolidated with those from Fields 1 to 4.

Sheep

In the first period of Trial 1 the sheep employed were Romney X Southdown cross lambs reared on the Station, but thereafter they were shorn Romney wether lambs purchased as top store lines from hill country where facial eczema rarely occurs. Check killings were made to determine that these lambs had no prior liver damage, and from their arrival at the Station they were held on brassica crops until required for the trials.

As the lines of lambs were remarkably even, division into groups by a process of restricted randomisation was considered unnecessary. When required, groups were drafted directly off the crops, ear-tagged and weighed, and allotted at random to experimental fields.

Pastures

Trials 1 and 2 were conducted on flat fields in which ryegrass and white clover composed at least 90% of the sward. It is characteristic of the Gisborne plain that species other than ryegrass and white clover do not persist under intensive sheep grazing, and in the present instance it is likely that the pastures were more uniform than would be the case elsewhere in the North Island of New Zealand.

During the summer and autumn well-distributed rainfall, in excess of normal, was experienced, and pasture production was usually above 40 lb dry matter per acre per day. This was surplus to the requirements of 10 lambs per acre, with the result that an appreciable quantity of fresh clippings was maintained on all the mown fields, while Field 10 became rank and stalky, with a high proportion of dead herbage.

The pastures in Trial 3 were also predominantly ryegrass and white clover, but as a result of prior differences in management were very dissimilar in height and proportion of dead and living herbage.

Mowing

Apart from the early period when a reel-type motor mower was used on Field 37, all mowing in these trials was carried out with a tractor-mounted rotary mower set to cut at 2½–3 in.

At the first cut on December 11 the fields in Trial 2 contained some rank growth which was wind-rowed, but at later cuts when new growth had taken place an even spread of clippings was obtained.

In general, this type of mower proved an extremely useful aid to experimental pasture control. It could be set accurately to cut to any desired height, and with it remarkably uniform swards were maintained.

Spore counting techniques

The washed grass sample method was applied as described by Thornton and Sinclair (1960). To obtain reasonably comparable dry matter estimates and to avoid any washing effect from heavy rain, care was taken to sample pastures when they were free from surplus moisture. When necessary, sampling was delayed for one or two days to obtain these conditions.

A spore trap was available from 5 January 1960, and for the first three weeks slides were prepared from 2 ft^3 air samples as recommended by Brook (1959). At this rate, however, slides prepared on some fields became so swamped with spores of the rust fungus *Puccinia coronata* Corda that identification of *P. chartarum* spores became virtually impossible. As a result, several trial runs were made, and it was found that ½ ft^3 was the maximum air sample which permitted reasonably accurate identification. Consequently, that volume was adopted as a routine from 28 January 1960, and, to increase the area sampled, duplicate slides were prepared from all fields under observation.

Counts made before 28 January were not strictly comparable to these later ones, but as far as could be judged they were all at a low level, and any errors in counting should not affect the overall accuracy of the results.

P. chartarum spores were evenly distributed over the 13 × 2 mm slide preparation. Therefore, to reduce the amount of work involved, only those along one 0.3 mm strip of the long axis were actually counted, spores per ft^3 being calculated from the sum of duplicate $\frac{1}{2}$ ft^3 samples $\frac{20}{3}$.

In operating the trap the following precautions were taken to reduce errors in sampling:

1. Approximately the same positions within each field were covered at each successive sampling.
2. The impactor orifice on the trap was screened by a bulb of fine wire mesh to prevent entry of grass seeds or fine pieces of litter.
3. Slides were prepared only when pastures were completely dry to the touch.
4. The trap was operated down wind when a strong wind was blowing.

Classification of liver lesions

All livers were examined macroscopically within two hours of slaughter and the severity of facial eczema lesions graded on a scale of 0 to 4, similar to that described by McFarlane *et al.* (1959).

It is characteristic of sub-clinical facial eczema in sheep that usually only portions of the liver, more commonly in the left lobe, are affected, and while it is recognised that any grading of these portions must be very subjective, it has been found possible to make a reasonably consistent estimate of the damage, provided the variable appearance of the lesions is known from experience.

Statistical analysis

Analyses were done by Dr H. R. Thompson, Applied Mathematics Laboratory, Department of Scientific and Industrial Research.

In calculating the correlations he used total spore trap counts with mean liver damage grades, and liveweight gains of each group of lambs for each period in Trials 1 and 2.

RESULTS AND DISCUSSION

Comparison of methods for estimating spore numbers

In Fig. 1 washed grass counts and spore trap counts from Field 10 (grazed only) and Field 37 (mown and grazed) in Trial 1 are shown for the whole season covering six lamb-grazing periods. Liver damage, where it occurred, is indicated by a histogram representing the mean grade for each group of lambs.

Analysis of the counts in Trial 1 showed that the two methods gave remarkably similar estimates of relative spore population. The correlations between spores per g dry matter and spores per ft^3 were as follows:—

Within Field 37—16 pairs: r = 0.98 ***.
Within Field 10—11 pairs: r = 0.60 (*).
Combined— 27 pairs: r = 0.99 ***.

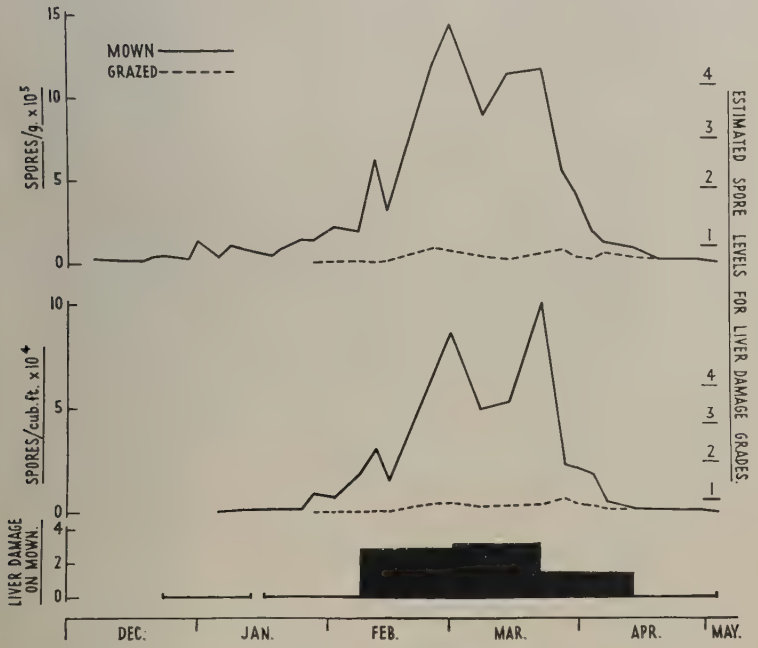

Fig. 1.—Spore numbers obtained by two methods in fields 37 (mown) and 10 (grazed only), during summer and autumn 1959-60. Upper lines show numbers of spores per g obtained by washing spores from pasture samples. Lower lines show numbers of spores per cu. ft. of air obtained by sampling with the spore trap. Mean amounts of liver damage suffered, during each of six successive periods, by lambs grazing in the mown field, are also shown. Spore numbers correlated with different degrees of liver damage are indicated.

Washed grass counts, when done on 10 samples per acre, give reproducible results. Thus, from a statistical viewpoint, there is little to choose between the methods. However, the collection and preservation of pasture samples for subsequent washing and counting would be impractical on a large scale. In contrast, the spore

*** Significant at 0.1%.
(*) Nearly significant at 5%.

TABLE 1. WEEKLY SPORE TRAP COUNTS ON UNIFORM 1 ACRE FIELD-GRAZED BY 10 LAMBS IN THREE SUCCESSIVE 4- WEEK PERIODS TOGETHER WITH THEIR LIVEWEIGHT GAINS AND INCIDENCE OF FACIAL ECZEMA

TRIAL 2

Period	Field No.	11	13	14	15	19	20	22	25	35
16 Jan. to 23 Feb.	Mean spore count per sample units of 10^3 per ft^3	25	17	11	7	8	2	2	2	11
	Mean liver damage grade	2.7	1.6	1.3	1.1	1.2	0.3	0.3	0.8	2.2
	No. of clinical cases	0	0	0	0	0	0	0	0	0
	Mean weight gain lb	10.8	15.6	17.4	18.9	13.2	16.9	14.4	11.6	9.2
23 Feb. to 22 Mar.	Mean spore count per sample units of 10^3 per ft^3	92	49	26	46	31	11	16	11	47
	Mean liver damage grade	3.9	3.9	2.6	2.4	1.7	0.9	1.2	2.1	3.9
	No. of clinical cases	8	2	0	1	0	0	0	0	5
	Mean weight gain lb	— 5.6	3.9	5.7	6.8	8.1	8.0	5.8	3.7	— 1.4
22 Mar. to 26 Apr.	Mean spore count per sample units of 10^3 per ft^3	29	17	8	8	14	7	9	6	19
	Mean liver damage grade	1.3	1.0	1.7	0.9	1.4	0.4	0.6	1.9	1.8
	No. of clinical cases	0	0	0	0	0	0	0	0	0
	Mean weight gain lb	10.7	11.3	13.1	11.8	12.2	16.0	12.0	14.4	12.0

trap, which is simply pushed around a pasture, takes less time, and prepares slides for counting in a dry, permanent state. It would clearly be the more simple and practical method for general field application.

Spore trap counts and liver damage

Mean numbers of spores per ft^3, mean liver damage grades, incidence of clinical facial eczema, and mean liveweight gains for all groups of lambs in the 3 periods of Trial 2, are summarised in Table 1. In addition, to illustrate seasonal trends of spore numbers in relation to liver damage, data from 3 fields are plotted in Fig. 2.

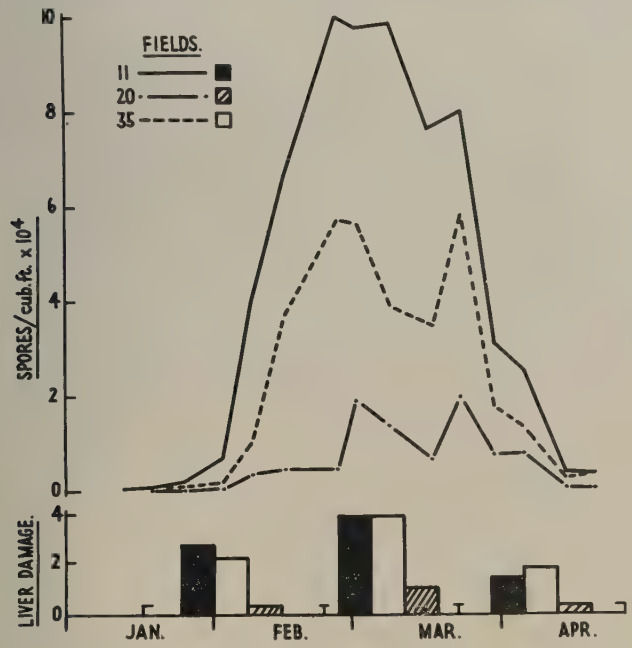

Fig. 2.—Spore numbers and liver damage in three fields. Spore number per ft^3 of air were obtained by sampling with the spore trap. Mean amounts of liver damage in each field are shown for each of the three periods 16 Jan.–23 Feb.; 23 Feb.–22 Mar.; 22 Mar.–26 Apr.

By combining these data with those from Field 37 in Trial 1, the correlation of total spore trap counts with mean liver damage grades during 31 separate grazing periods was: $r = 0.85$ (significant at 0.1%). From the regression equation of liver damage on spore counts the mean spore numbers in individual samples associated with the various grades of liver damage were calculated as follows:

TABLE 2. WEEKLY SPORE TRAP COUNTS FROM SIX DIFFERENT PASTURES IN TRIAL 3, TOGETHER WITH LIVER DAMAGE GRADES IN LAMBS GRAZING IN ONE PERIOD FROM JANUARY TO MAY

Place	Description of Pasture at 1 February	Spores per ft³ in units of 10^3											Liver damage grade
		To 2 Feb.	9 Feb.	15 Feb.	26 Feb.	1 Mar.	8 Mar.	16 Mar.	23 Mar.	30 Mar.	5 Apr.	After 5 Apr.	
Manutuke 1	2–3 in. high, green, little dead herbage	< 1	1	1	1	2	1	2	1	1	1	< 1	0.0
Manutuke 2	2–3 in. high, green, some dead stalk	< 1	2	1	3	5	2	1	2	1	1	< 1	0.0
Manutuke 3	4–8 in. high, very stalky	< 1	1	1	3	4	1	2	2	1	1	< 1	0.0
Manutuke 4	6–12 in. high, rank, > 50% dead herbage	< 1	1	1	7	3	3	1	2	1	1	< 1	0.0
Farm 1	4–6 in. high, green, dominant white clover	< 1	2	3	8	6	4	6	4	1	1	< 1	0.0
Farm 2 *	4–8 in., eneven, high dead herbage	< 1	14	3	19	30	15	13	20	9	2	< 1	1.1

* Mown and cleaned up with cattle in early February. Became similar to farm 1.

Liver damage grade 1 = 6 × 10^3/ft^3 (spore trap) or 1.0 × 10^5/g. (washed grass)

Liver damage grade 2 = 24 × 10^3/ft^3 (spore trap) or 4.3 × 10^5/g. (washed grass)

Liver damage grade 3 = 42 × 10^3/ft^3 (spore trap) or 7.5 × 10^5/g. (washed grass)

Liver damage grade 4 = 60 × 10^3/ft^3 (spore trap) or 10.8 × 10^5 g. (washed grass)

These levels obviously have a limited meaning, as they are based on a particular period of grazing. They give no indication of the liver damage which would have occurred had lambs been grazed for longer periods on the pastures, as would be likely to occur under normal farming conditions.

However, the upper and lower levels indicate the range of spore numbers beyond which pastures are either safe or extremely toxic. For example, on Field 37 in Trial 1, and on Fields 11, 13, 15, and 35 in Trial 2, where spore numbers approached or exceeded the Grade 4 level of 60 × 10^3 per ft^3 during the period ended 22 March, 84% of lambs suffered gross liver damage and 32% developed clinical facial eczema.

The results from 5 fields in Trial 3, shown in Table 2, and from Field 10 in Trial 1, demonstrate the other extreme. On these fields, with minor exceptions, spore numbers did not exceed the grade 1 level of 6 × 10^3 spores per ft^3, and all livers were normal irrespective of the duration of grazing periods or of the height and quality of the pastures.

From a practical viewpoint the most important aspect of facial eczema control is to be able to determine when pastures are safe for grazing sheep. In this context, spore numbers below the grade 1 level should provide a useful index.

Spore trap count and liveweight gains

Although not strictly relevant to this study, the opportunity was taken to correlate spore trap counts and liver damage with liveweight gains as the data were available (Table 1).

Using pairs from the same 31 periods as in the previous section, the following correlations were calculated:—

1. Spore trap counts with liveweight gains: $r = -0.76$ ***.
2. Liver damage grades with liveweight gains: $r = -0.80$ ***.

Thus, in fields where the quality of pasture was uniform, the wide between-field and between-period variation in liveweight change, ranging from +0.5 lb to —0.2 lb per day, could be explained by the varying incidence of *P. chartarum* spores. The second correlation suggests that the amount of fungus ingested by the grazing lambs affected their

*** Significant at 0.1%.

rate of growth by interference with their liver function. This occurred independently of the weight loss caused by clinical facial eczema; for when the data from Field 11, where most clinical cases occurred, were removed from the calculations, the correlation remained significant at 0.1%.

These observations suggest that the role of *P. chartarum* in the syndrome of so-called autumn flush ill-thrift of hoggets may be more important than has hitherto been considered.

GENERAL DISCUSSION

The results given here amplify those set out by Thornton and Sinclair (1960). The high correlation obtained in the present work between numbers of spores of *P. chartarum* in a pasture and the severity of liver damage in sheep grazing there, suggests that this fungus is, in fact, the major or sole cause of facial eczema in the field. It is desirable, therefore, to relate these results to the problem of practical facial eczema control, but when endeavouring to do so the two opposing viewpoints must be considered.

On the one hand, the strong correlation between spore numbers and degree of facial eczema suggests that spore trap counts could provide a more precise basis for facial eczema warning than the particular set of climatic conditions suggested by Mitchell *et al.*

On the other hand, the wide between-field variation in spore numbers, which made this correlation possible, indicates that spore counts could have a very limited meaning on a district basis. For example, in Trial 2, Field 11 became highly toxic, while Field 20 remained relatively safe. If this variation can occur between apparently identical pastures on the same soil type and geographical aspect, then it is reasonable to assume that the variation within a district would be very much greater, with the result that spore counts could give no reliable indication of facial eczema danger unless carried out on an impossibily large scale.

However, it is possible to reconcile these opposing views by considering seasonal trends, rather than absolute levels, in spore numbers. When the data from all trials are examined together it will be seen that, in spite of the wide variation in maximum counts, the seasonal pattern was similar. From their original low levels, counts on all mown fields (including the field on Farm 2 which was mown at a critical time between 2 and 9 February) increased to their respective maxima between 2 February and 1 March, and all declined simultaneously after 23 March. To a lesser extent the same pattern was apparent on the fields in Trials 1 and 3, which were not mown. Thus, under a wide range of conditions, active growth of *P. chartarum* took place during the same period, which was clearly delineated by the spore trap. All the data from trials at Manutuke in 1959 and 1960 suggest that this one defined period of intensive spore production is a growth characteristic of *P. chartarum,* and although it can be altered by the artificial conditions created by regular mowing and spreading of fresh clippings, it is likely that within any district covered by the same climatic conditions

the period would remain constant from farm to farm. In consequence, spore trap counts from a series of representative pastures within the district, while expected to vary widely and not to define any particular toxic area, would define the period when intensive spore production took place and when some pastures were almost certain to become toxic. The main advantage would be that when spore counts did not increase to a dangerous level following the onset of appropriate climatic conditions, unnecessary facial eczema warnings could be avoided. Conversely, when there was a general upward trend in spore counts, warnings could be held in force until the characteristic decline took place.

To test these assumptions, more extensive use of the spore trap under field conditions appears warranted.

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REFERENCES

Brook, P. J. 1959: A volumetric spore trap for sampling pastures. *N.Z. J. agric. Res. 2:* 690–3.

McFarlane, D.; Evans, J. V.; Reid, C. S. W. 1959: Photosensitivity diseases in New Zealand. XIV. The pathogenesis of facial eczema. *Ibid. 2:* 194–200.

Mitchell, K. J.; Walsh, T. O.; Robertson, N. G. 1959: Weather conditions associated with outbreaks of facial eczema. *Ibid. 2:* 584–604.

Percival, J. C. 1959: The association of *Sporidesmium bakeri* with facial eczema. *Ibid. 2:* 1041–56.

Thornton, R. H.; Percival, J. C. 1959: A hepatotoxin from *Sporidesmium bakeri* capable of producing facial eczema disease in sheep. *Nature, Lond. 183:* 63.

Thornton, R. H.; Sinclair, D. P. 1960: Some observations on the occurrence of *Sporidesmium bakeri* Syd. and facial eczema disease in the field. *N.Z. J. agric. Res. 3:* 300–13.

EFFECTS OF INITIAL CONSOLIDATION ON SILAGE

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ABSTRACT

Mixed dairy pasture harvested at three stages of growth from the same area was ensiled using three degrees of consolidation in small experimental silos. Better fermentation characteristics, lower dry matter losses, and higher digestibilities were associated with heavier consolidations and with less mature pasture.

INTRODUCTION

It has long been considered (Watson 1939) that some aeration in the initial stages of ensilage is necessary in order to promote the desired fermentation. In silage-making practice in Britain and New Zealand the rates of filling and consolidation are regulated to permit aeration of the ensiled mass to the extent that temperatures are usually held within the range 27°C to 38°C.

The required degree of consolidation is thought to depend on the maturity of the ensiled herbage, less being necessary for immature herbage which compacts readily. Excessive consolidation is deemed to promote anaerobic conditions at too early a stage of the fermentation.

As the literature appeared to contain little experimental support for these views, it was considered desirable further to investigate optimum physical conditions for ensilage. The present experiment was designed to obtain information on the role of consolidation in the silage fermentation.

EXPERIMENTAL

Herbage ensiled

The area from which the grass was harvested was closed to stock on 12 September 1957. The herbage was cut from different sections of the area on 17 October (M1)*, 29 October (M2), and 13 November (M3). The sward comprised mainly perennial ryegrass (*Lolium perenne* L.), with white clover (*Trifolium repens* L.) distributed

throughout. Some cocksfoot (*Dactylis glomerata* L.) and other grasses were present. The first-cut material was leafy, with little stem development; the second-cut was still leafy, but considerable stem development had occurred; while the third harvest contained much ryegrass in flower.

TABLE 1. DESCRIPTION OF ENSILED HERBAGES

Maturity	M1	M2	M3
Date ensiled	17-10-57	29-10-57	13-11-57
Growth stage	Vegetative	Vegetative	Flowering
Dry matter %	19.2	17.3	20.4
Soluble carbohydrates (ethanol)	11.1	11.8	9.8
Soluble carbohydrates (water)	3.9	3.5	3.6
Crude protein	18.8	16.7	12.4
O.M. digestibility	82	76	69

Chemical composition of the herbages is summarised in Table 1. It was not convenient to conduct digestibility trials, but approximate organic matter digestibility values were calculated from lignin concentrations using a formula given by Lancaster (1944).

Ensiling procedure

The herbage, cut with a sickle-bar mower, was immediately ensiled under three degrees of consolidation in 6 ft high by 3 ft diameter concrete pipe silos of the type described by Barnett (1954). Two silos were used for each consolidation treatment on each of the three cutting dates. During filling one thermo-couple was placed in the centre of each silo.

*"Heavy consolidation" (C3)**. This was achieved by continuous trampling as filling proceeded until about 1,000 lb of material was ensiled. The grass was immediately covered with a .005 in. polythene sheet, and weighted with a 500 lb concrete disc.

"Medium consolidation" (C2). This was obtained by occasionally trampling so that 800 lb of material was filled into each silo. Covering and weighting were delayed for one day to permit some diffusion of air into the ensiled herbage.

"Light consolidation" (C1). No trampling was employed until filling was nearly complete when sufficient trampling was imposed to fill the silos with 600 lb of grass. Covering and weighting were delayed for two days.

* M1, M2, and M3 refer to stages of maturity; C1, C2, and C3 refer to degrees of consolidation. (See Table 2.)

TABLE 2. INITIAL MEAN DENSITIES (lb/ft³), MAXIMUM TEMPERATURES (°c), AND DAYS ENSILED

Consolidation	Ensiled maturity	17-10-57 M1		29-10-57 M2		13-11-57 M3	
Light C1	Silo No.	1	2	7	8	17	18
	Density	14.3	14.2	13.3	13.8	12.1	11.9
	Temp.	*	38	42	41	50	56
	Days ensiled	186	230	174	238	203	244
Medium C2	Silo No.	3	4	9	10	13	14
	Density	19.2	19.1	18.6	18.9	16.7	17.0
	Temp.	25	26	28	30	37	33
	Days ensiled	186	230	195	238	180	123
Heavy C3	Silo No.	5	6	11	12	15	16
	Density	24.0	24.2	21.8	22.1	21.6	21.6
	Temp.	26	24	25	28	27	27
	Days ensiled	186	230	195	238	180	244

* No data available.

In Table 2 these arbitrary consolidations have been described in terms of average densities calculated from grass weights and silo volumes.

After one week, weights and covers were removed, a sacking marker was laid on top of the ensiled material, and a further 200 lb of fresh-cut pasture were added to each silo which was then covered and weighted as before, and finally covered with a water-tight metal lid. The additional grass was used as a sealing material only, and was not considered in subsequent measurements.

Sampling of grass

The herbage was weighed into the silos in 25 lb lots, 200 lb being placed in turn in each of the six silos allocated to each cut, the process being repeated until each silo was filled. As each 25 lb lot of grass was weighed, a 1–2 lb sample was set aside. When eight such samples had been taken they were mixed and sub-sampled as follows:

(a) *For dry matter determinations:* three 200–300 g samples were weighed in metal trays and dried at 100°c for 18 hours in forced draught.

(b) *For soluble sugars:* one 500 g sample was stored in a polythene bag at —15°c. The 24 samples thus accumulated were randomly bulked in sets of 6 for analysis.

(c) *For other chemical analyses:* 100 g samples were thinly spread on open mesh cloth trays and dried to millable condition in forced draught at 40°c. These were composited and milled to pass a 60-mesh sieve.

Processing of silages

For practical convenience, and since little further change was expected in the ensiled material after six months of storage, the silos were opened in sets of four at 21-day intervals commencing 21 April 1958. After removal of the sealing layer, the material from each silo was weighed in 200 lb lots, from which a 4–5 lb sample was removed. From this sample, four 300 g lots were weighed for dry matter determinations by the same procedure as for the grass. One 500 g sample was stored in a polythene bag at —15°c for subsequent chemical analysis.

Digestibility trials

After removal of the samples, the remainder of the contents of each silo was weighed out for a digestibility trial. Three sheep were used in a trial which comprised a 7-day pre-period and a 14-day test period. Sixty-three 2 kg rations were prepared from the contents of each silo. The rations were placed in polythene bags from which the air was forced, before sealing with a rubber band. Sets of 12 such bags containing the daily rations of each sheep in the four concurrently-run digestibility trials were sealed in a heavy-gauge polythene bag, and stored at room temperature (2°c–15°c).

Chemical analyses

Herbage was analysed as follows:

1. Total N (Kjeldahl) on 100–200 mg of dried milled herbage.
2. Lignin on 1 g milled sample using a 72% H_2SO_4 method as modified by Lancaster (1944).
3. Carbohydrates soluble in 80% ethanol and in cold water were determined in 20 g samples of chopped frozen herbage by the ferricyanide procedure (Fujita and Iwatake 1932, following deproteinisation with cadmium (Doak 1939), and hydrolysis with 0.1 N H_2SO_4 at 100°c for 15 minutes.

Silage was analysed as follows. The frozen samples were chopped and minced through a sausage machine. An extract of the sample was prepared by macerating 40 g of minced silage with 400 ml cold distilled water for 5 minutes and pouring through a fine-holed Buchner funnel. After a half hour settling period, the extract was used for chemical analysis.

The following determinations were made:—

A. In extracts

1. Volatile N (VN) by the method of Conway (1947) on 1 ml of extract.

2. Steam volatile acids (SVA) by distillation of 5 ml of extract through a Markham still (McClymont 1951).

3. Lactic acid by the ceric sulphate method of Elsden and Gibson (1954), after clearing 150 ml of extract by the lime-copper method (Barnett 1954).

B. In minced silage

1. Dry matter by drying 2 × 100 g samples in a ventilated oven at 100°c for 18 hours.

2. Total N in three 8–10 g samples using the procedure as for grass.

3. pH on juice expressed from silage after the addition of an equal weight of water.

Statistical analysis (Tables 8 and 9)

The design of the experiment was a 3 × 3 factorial with two replicates (silos) for each of the 9 treatment combinations. In the analysis of variance, the main effects of each treatment, maturity (M) and consolidation (C) were partitioned into a linear (l) and a quadratic (q) component. (See, for example, Cochran and Cox 1950). The former reflects the difference between the extreme levels, the latter the difference between the intermediate level and the mean of the other two. The interaction effects were correspondingly partitioned.

In the case of digestibility determinations, the effect of separate weeks in the trial fortnights, on the one hand, and of individual silos, on the other, were also studied using analysis of variance procedures. Finally regression and covariance analyses were used to study and allow for possible association of time ensiled with the various characters investigated.

TABLE 3. DRY MATTER CONTENT AND LOSS

	Dry matter in silages (%)				Dry matter loss (%)			
	M1	M2	M3	Means	M1	M2	M3	Means
C1	16.64	15.13	17.53	16.43	37.2	38.3	33.5	36.3
C2	17.96	15.32	17.14	16.80	28.4	33.7	32.7	31.6
C3	19.11	15.71	17.65	17.49	17.4	28.3	19.9	21.9
Means	17.90	15.38	17.44	16.91	27.6	33.4	28.7	29.9

RESULTS

The treatment sub-class means are presented in Tables 3–7, and the analyses of variance are summarised in Tables 8 and 9.

Dry matter content (Table 3)

The dry matter content of silage from medium maturity grass was significantly lower than that of the other two silages, which

did not differ significantly. There was a significant linear increase with increasing consolidation; however, the difference between the values for heavy and light consolidation declined significantly with advancing maturity (2.47, 0.58, 0.12), i.e., there was some interaction.

TABLE 4. DIGESTIBILITY OF DRY MATTER AND NITROGENOUS MATTER

	D.M.				N			
	M1	M2	M3	Means	M1	M2	M3	Means
C1	65.6*	62.1	53.9	60.6	65.8	64.2	61.2	63.7
C2	69.7	62.4	56.2	62.8	70.0	68.0	61.4	66.5
C3	76.3	67.3	63.3	68.9	76.3	71.9	68.5	72.2
Means	70.6	63.9	57.8	64.1	70.7	68.0	63.7	67.5

* Each value is derived from 14 day estimates with 6 sheep per treatment.

Dry matter loss (Table 3)

Losses in the medium maturity treatment were significantly greater than in the early and later maturity groups, which did not differ significantly. There was a highly significant linear decrease with increasing consolidation, and no interaction.

Lactic acid (Table 6)

Although both maturity and consolidation exerted a substantial influence on lactic acid concentration, these effects were non-significant owing to the strong interaction between consolidation and muturity. Thus the difference between heavy and light consolidation (8.69) was very much greater for the first cut than for the second (3.53) and third cuts (3.01), which differed but little from one another.

Steam volatile acids (Table 6)

The effect of maturity on SVA concentrations was highly significant but non-linear, M2 being the greatest value. On the other hand, SVA declined steadily with increasing consolidation. Significant interaction with stage of maturity was, however, manifested by the significant linear decrease of the differences C1–C3 (5.42, 3.08, 0.96).

pH (Table 6)

The least mature herbage resulted in silages with significantly lower pH values as compared with the other two groups which did not differ. Increasing consolidation was associated with an approximately linear decrease in pH. However, the effect was dependent on stage of maturity, the differences C1–C3 declining significantly with maturity (1.00, 0.38, 0.17).

TABLE 5. DRY MATTER DIGESTIBILITY

(a) Weekly estimates—duplicate silos

	Week 1				Week 2			
	M1	M2	M3	Means	M1	M2	M3	Means
C1	67.2*	62.9	53.0	61.0	63.8	61.3	54.5	59.8
C2	70.3	62.4	54.4	62.4	69.2	62.3	58.0	63.2
C3	76.0	69.3	65.0	70.1	76.5	64.9	61.2	67.5
Means	71.2	64.9	57.5	64.5	69.8	62.8	57.9	63.5

* Each value is the mean of weekly estimates using 6 sheep (3 sheep per silo).

(b) 14-day estimates—single silos

	Silo A				Silo B			
	M1	M2	M3	Means	M1	M2	M3	Means
C1	65.6*	62.9	54.4	61.0	65.3	61.2	53.1	59.9
C2	71.2	63.5	59.2	64.6	68.2	61.2	53.1	60.9
C3	76.7	67.8	64.6	69.7	75.8	66.4	61.6	68.0
Means	71.2	64.7	59.4	65.1	69.8	63.0	55.9	62.9

* Each value is the mean of 14-day estimates using 3 sheep.

TABLE 6. pH, LACTIC ACID, AND STEAM VOLATILE ACIDS

	pH				Lactic acid % DM				Steam volatile acids % DM			
	M1	M2	M3	Means	M1	M2	M3	Means	M1	M2	M3	Means
C1	5.06	5.23	5.15	5.15	1.43	0.83	0.55	0.94	8.52	10.30	8.57	9.13
C2	4.70	5.14	5.11	4.98	5.19	1.71	0.81	2.57	6.05	10.36	9.64	8.68
C3	4.06	4.85	4.98	4.63	10.12	4.36	3.56	6.02	3.10	7.22	7.61	5.98
Means	4.61	5.07	5.08	4.92	5.58	2.30	1.64	3.17	5.89	9.29	8.61	7.93

Total N (Table 7)

The decline in nitrogen content with advancing maturity was highly significant and approximately linear. The effect of consolidation was just significant, the values associated with heavy consolidation being lower than those for light and medium, which did not differ. There was no consolidation—maturity interaction.

Volatile N (Table 7)

The value for volatile nitrogen was significantly affected by maturity, the second cut material giving the highest value. Increasing consolidation led to a linear decline in the values, but a significant decrease in this effect with maturity is indicated by the linear decrease of the differences C1–C3 with advancing maturity (11.5, 8.8, 4.4).

Digestibility coefficients (Table 4)

The effects of both maturity and consolidation on the digestibility of dry matter and of nitrogen were highly significant, and the interaction between these factors appeared negligible. Further analysis of the treatment main effects suggests that over the ranges considered they were substantially linear, i.e., the intermediate level of either factor led to digestibilities about midway between those for the two extremes. However, for dry matter digestibility the increase from medium to heavy consolidation (6.1 units) was greater ($P \doteqdot 1/15$) than that from light to medium (2.2).

TABLE 7. TOTAL NITROGEN AND VOLATILE NITROGEN

	Total N % DM				Volatile N % total N			
	M1	M2	M3	Means	M1	M2	M3	Means
C1	3.84	3.48	2.96	3.43	23.7	31.2	26.0	27.0
C2	3.73	3.62	2.98	3.44	19.4	25.6	26.5	23.9
C3	3.46	3.20	2.78	3.15	12.2	22.4	21.6	18.8
Means	3.68	3.44	2.90	3.34	18.5	26.4	24.7	23.2

Effects of storage time (Table 5a, b)

More detailed examination of the dry matter digestibility data reveals that, in addition to the highly significant overall treatment effects, the variation between silos within the same treatment was also highly significant. The digestibility of material from silos that were opened first exceeded that from the silos opened later, in each pair, by an average of 2.2 units. In seven cases the difference in storage time was 41–44 days, and in the remaining two it was 64 days (Table 2). With these small variations in storage time it was not possible to establish any satisfactory relation between storage time and digestibility.

Inspection of the weekly digestibility data shows the variation between weeks to be highly significant, the digestibility in the first week being on average one unit higher than in the second week. Exceptions to this are seen in treatments M3C1 (53.0 V 54.5), M3C2 (54.4 V 58.0), and M1C3 (76.0 V 76.5).

TABLE 8. ANALYSIS OF VARIANCE—CHEMICAL COMPOSITION AND DIGESTIBILITY (mean squares only)

Source of variation		df	DM	DM loss	Lactic acid	Steam-volatile acids	pH	Total nitrogen (N)	*Volatile N* Total N	DM (1) digesti-bility	Nitro-gen (1) digesti-bility
Maturity	Linear (M_l)	1	.63	3.1	46.56	22.15	.678	1.778**	116.6	487.7**	146.3**
	Quadratic (M_q)	1	20.92**	112.0*	6.88	16.76**	.209*	.086	93.7	.3	2.8
Consolidation	Linear (C_l)	1	3.35	626.4**	77.32	29.78	.806	.238*	200.9	211.4	218.4**
	Quadratic (C_q)	1	.10	24.8	3.31	5.12†	.036	.097†	4.0	15.7*	8.9
Interaction	$M_l \times C_l$	1	2.75**	18.9	16.11**	9.91**	.352**	.021	25.6**	.8	5.0
	$M_l \times C_q$	1	.19	16.2	.30	1.14	.006	.001	1.0	.9	3.6
	$M_q \times C_l$	1	.33	29.7	3.59*	.01	.029	—	.4	15.8*	1.0
	$M_q \times C_q$	1	.01	8.8	—	.45	—	.031	9.4*	.2	4.2
	Odd vs even	1	2.46**	6.1	.12	.33	.024	.027	7.5†	18.8**	12.6
	Residual	8	.17	12.1	.50	.72	.014	.036	1.8	1.7	9.3
	Total	17	1.89	55.5	9.51	5.38	.133	.151	27.9	45.0	28.1

(1): Fourteen-day estimates, means of three sheep (cf. Table 9)
df: degrees of freedom
†: $P < .10$
*: $P < .05$
**: $P < .01$

In general, therefore, it may be concluded that the dry matter digestibility of silage was still declining after 6–8 months in storage. This result is of some importance in itself; however, in terms of the present investigation, the possibility exists that differences in storage times may have influenced treatment differences.

Of the other observations made, only dry matter per cent and volatile nitrogen showed consistent differences between pairs of silos. In all nine treatment pairs the first silages opened contained the lower dry matter percentage. The mean difference 0.74 was highly significant. In six cases VN was greater in the first silages opened. The differences approach significance, the overall mean difference being 1.3. The data are inadequate to establish any relation between these factors and ensiling time. However, for both DM% and VN, these conclusions are little altered when allowance is made by covariance analysis for the effect of ensiling time.

TABLE 9. ANALYSIS OF VARIANCE—WEEKLY DIGESTIBILITY ESTIMATES (mean square only)

Source of variation	df	DM digestibility
Treatments (see Table 8)	8	553**
Replicates (Odd v. even)	1	131**
Residual	8	9.0
Sheep (within silos)	36	9.6
Weeks (1st v. 2nd)	1	26.3
Weeks x Treatments	8	21.4**
Weeks x Replicates	9	9.0
Weeks x Sheep (within silos)	36	6.0
Total	107	51.1

DISCUSSION

The decline with advancing maturity in total N and in digestibility of dry matter and nitrogen of herbage presumably reflects corresponding changes in the ensiled material. The influence of maturity on the other factors measured, however, is not so readily explainable. Silages derived from M1 show the most desirable characteristics in all items measured; those from M2 show the least desirable effects, except that pH values were similar in M2 and M3 silages. An explanation of this situation must be sought in some character of the ensiled herbage not necessarily associated with maturity. For this purpose the descriptions of the herbages provided in Table 1 are inadequate, though the lower dry matter content of M2 is postulated as a contributing factor.

The influence of maturity on consolidation effects is shown by the interactions in the cases of dry matter per cent, lactic acid, SVA, pH, and VN. These interactions do not support the contention that consolidation should be controlled on the basis of maturity: rather is it evident that the heaviest consolidation gave the most favourable

result with each herbage tested and that the least mature material gave the greatest response to consolidation treatment. The linearity of the consolidation effects (as far as it can be determined from three points) suggests that optimum conditions for ensilage do not necessarily lie within the range of the treatments imposed, and may well occur at consolidations greater than C3. This suggestion is supported by the results of Cordukes *et al.* (1959), which demonstrated that dry matter losses were least when initial densities of ensiled material were highest. The maximum initial density employed was 42 lb per cu. ft., considerably greater than the highest density attained in the present study.

The results of the present experiment are in harmony with those of Langston *et al.* (1958) and of McDonald *et al.* (1960). Langston and his co-workers showed that the efficiency of the silage process, measured in terms of the chemical nature of the silage and losses of nutrients, was greater when anaerobic conditions were established rapidly. McDonald and his group showed the more efficient ensiling process to be associated with greater initial densities and greater applied pressures.

The interpretation of experiments involving different degrees of consolidation is complicated by the interrelation of several contributing factors. At least three variables may contribute to the effects observed: (1) the initial density; (2) the amount of aeration; and (3) the resulting temperature. That temperature *per se* plays a decisive role in the silage fermentation was demonstrated by Nilsson *et al.* (1956); their data showed optimum temperatures to be less than 24°C. The effects of density and of aeration have not been adequately investigated.

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REFERENCES

Barnett, A. J. G. 1954: "Silage fermentation." p. 146. Butterworth's Scientific Publications, London. 208 pp.

Cochran, W. G.; Cox, Gertrude M. 1950: "Experimental designs." pp. 56 and 135. Wiley, New York. 454 pp.

Conway, E. J. 1947: "Microdiffusion analysis and volumetric error." 2nd ed. pp. 85–7. Crosby Lockwood, London. 357 pp.

Cordukes, W. E.; Shearer, D. A.; Cooper, D. J. 1959: *Canad. J. Plant Sci. 39:* 127–34.

Doak, B. W. 1939: *N.Z. J. Sci. Tech. 21, 2B:* 90–5.

Elsden, S. R.; Gibson, Q. H. 1954: *Biochem. J. 58:* 154–8.

Fujita, A.; Iwatake, D. 1932: *Biochem. Z. 242:* 43.

Lancaster, R. J. 1944: *N.Z. J. Sci. Tech. 25, 4A:* 137–51.

Langston, C. W.; Irvin, H.; Gordon, C. H.; Bouma, C.; Wiseman, H. G.; Melin, C. G.; Moore, L. A.; McCalmont, J. R. 1958: *U.S.D.A. Bull. 1187.* 73 pp.

McClymont, G. L. 1951: *Aust. J. Agric. Res. 2:* 158–80.

McDonald, P.; Stirling, A. C.; Henderson, A. R.; Dewar, W. A.; Stark, G. H.; Davie, W. G.; Macpherson, H. T.; Reid, A. M.; Slater, J. 1960: *Edinburgh School of Agriculture Tech. Bull. 24:* 83 pp.

Nilsson, R.; Toth, L.; Rydin, C. 1956: *Arch. Mikrobiol. 23:* 366–75.

Watson, S. J., 1939: "The science and practice of conservation". 1st ed. Vol. 1. pp. 151–2. Fertil. Feed. St. J., London. 415 pp.

SOME FACTORS AFFECTING THE PERSISTENCY OF SHORT-ROTATION RYEGRASS

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ABSTRACT

A study was made of the extent of stem elongation and seed-head emergence occurring in short-rotation ryegrass pastures during the reproductive period of growth. Pastures under both lax and continuous grazing treatments were compared. Data for short-rotation ryegrass were compared with those obtained for perennial ryegrass.

For both ryegrass varieties tiller numbers per unit area were significantly higher in the continuously-grazed pastures, while for both grazing treatments tiller numbers were significantly higher in the perennial ryegrass stands.

The percentages of tillers with elongated stems and emerged seed-heads were highest in the short-rotation ryegrass pastures, particularly under lax grazing. These higher percentages occurred later than in the perennial ryegrass pastures, although the period over which elongation and emergence occurred was more prolonged.

These results are discussed in relation to: (1) the persistency of short-rotation ryegrass in pastures, particularly where hard grazings are practised during the drier summer months; and (2) changes in the genotypic structure of the variety under different systems of pasture management.

INTRODUCTION

Previous work (Brougham 1960; Brougham *et al.* 1960) has shown that the persistency of short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*) in pastures is adversely affected by hard grazing during the summer and early autumn. At other seasons hard grazing shows little effect.

Branson (1953) showed that the persistency of rangeland species was related to both: (1) the positions of the growing points of stems relative to grazing heights; and (2) the ratio of flower stalks to vegetative stems during the reproductive period of growth. The work reported in this paper was carried out to investigate the possibility that the lack of persistency of short-rotation ryegrass in pastures under hard summer grazing could also be related to these factors. For comparison, data were also obtained for perennial ryegrass (*Lolium perenne* L.) because of its known persistency in pastures under hard summer grazing.

EXPERIMENTAL

The work was carried out at Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, during the spring, summer, and early autumn of 1960–1. The pastures studied were pure stands of short-rotation and perennial ryegrass fertilised with superphosphate and sulphate of ammonia. Each was autumn-sown in 1959.

For each variety one pasture was rotationally and the other continuously grazed with sheep. In the rotationally-grazed stands the height of herbage at each grazing was between 9 in. and 15 in., and the height of herbage left after grazing was between 3 in. and 4 in. Following grazing, these pastures were topped with a sickle-bar mower to remove stems uneaten above the 3–4 in. level. In the continuously-grazed swards the herbage height ranged from 3–6 in. Hereafter these treatments will be referred to as lax and continuous grazing respectively.

At 3 or 4 week intervals during the period of study the herbage in 5 or 6 randomly sited quadrats (each of 1 sq. ft.) in each treatment was cut to ground level, then sub-sampled in the laboratory, on a greenweight basis, to approximately one fifth of the total greenweight after removal of dirt and litter. The tillers present in each sub-sample were then classified into groups on the basis of variation in distance of the growing point from the base or cut end of each tiller. From these groupings estimates were made of the total number of tillers per unit area of pasture, of the percentages of these tillers with growing points 1 in. and 3 in. above the base or cut end of each tiller, and of the percentage with emerged seed-heads. The numbers of axillary tillers originating from the nodes of elongating stems were also determined but were not included in the estimates of tiller populations per unit area. Over-ripe and dead seed-heads were removed before the samples obtained from the continuously-grazed pastures were analysed.

To measure the effect of hard defoliation during the summer on the persistency of short-rotation ryegrass, areas of the laxly-grazed pasture were cut to $\frac{1}{2}$ in. from ground level on 27 January 1961, following an overall grazing to 3 in. Subsequently all areas were grazed on 23 February and 16 March. At each of these grazings, 3–4 in. of herbage were left, except on those areas that had been cut hard in January, where less than 1 in. of herbage remained. The tiller populations of these areas were estimated from counts of randomly sited sq. ft. quadrats on 6 April 1961.

RESULTS

Fig. 1 shows the changes recorded in tiller populations per unit area for the four pastures at successive samplings.

Of interest are the significantly higher numbers of tillers per unit area in the continuously-grazed pastures of both ryegrass varieties, and the marked decreases (approximately 50 per cent)

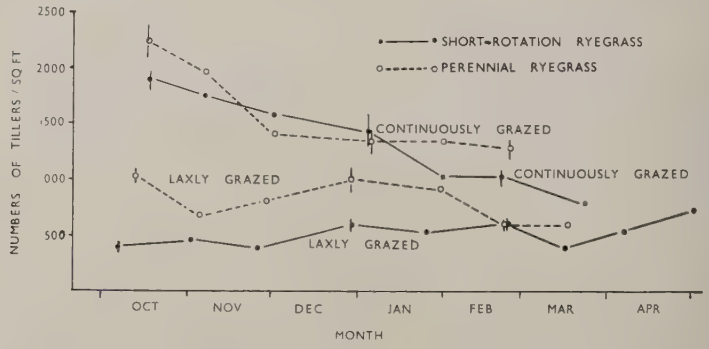

Fig. 1.—Tiller numbers in the four pastures at each sampling. Limits (± S.E.) are shown for 3 selected dates.

recorded in these pastures compared with the laxly-grazed pastures over the period of study. Under both systems of management, tiller populations in the perennial ryegrass pastures were generally higher than those in the short-rotation ryegrass pastures. These changes in tiller populations can be explained in part by the results presented in Figs. 2 and 3.

Fig. 2 shows the percentages of tillers at each sampling with growing points above 1 in. and 3 in. from the base or cut end.

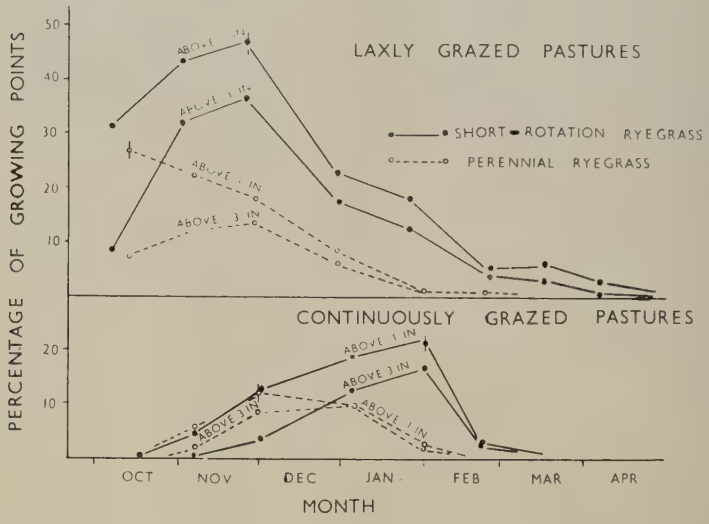

Fig. 2.—Percentages of tillers with growing points above two levels from the base of tillers in the four pastures at each sampling. Maximum values of limits (± S.E.) are shown for the 1 in. level.

The important features of Fig. 2 are:—

(1) The percentages of tillers in which stem elongation occurred were significantly greater in the short-rotation ryegrass pastures under both systems of grazing than in the corresponding perennial ryegrass pastures.

(2) Under both systems of grazing the peak time of stem elongation occurred earlier in the perennial ryegrass pastures.

(3) The period over which stem elongation occurred was more prolonged in the short-rotation ryegrass pastures.

(4) Stem elongation in both ryegrass varieties was delayed under continuous grazing.

These features are reflected in Fig. 3, where the percentages of tillers with emerged seed-heads are shown for the four pastures at each sampling.

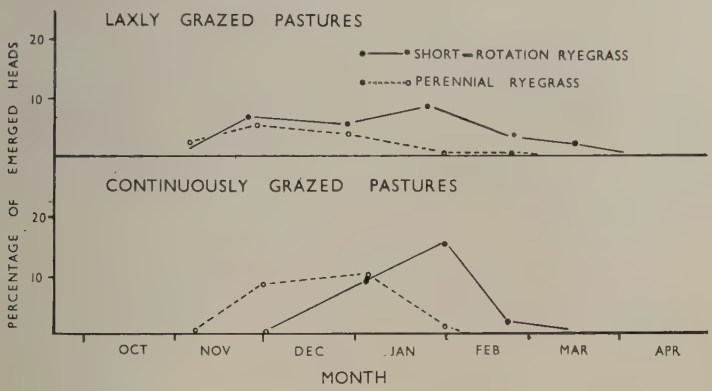

Fig. 3.—Percentages of tillers with emerged seed-heads in the four pastures at each sampling.

In addition, the results shown in Fig. 3 illustrate that the period of seed-head development in short-rotation ryegrass pastures is more prolonged than in perennial ryegrass pastures.

A considerable amount of axillary tiller development occurred, in all except the continuously-grazed perennial ryegrass pasture, during the period of stem elongation. However, because of their position in relation to ground level, marked variations in numbers occurred at successive samplings, owing to removal and damage at grazing and fluctuations in weather during the drier summer months. For these reasons they were not included in the tiller population estimates shown in Fig. 1. In general, the period of peak axillary tiller development was associated with the development of seed-heads, and the largest numbers were recorded at the samplings where the highest mean numbers of emerged seed-heads were present.

The effect of hard grazing during the summer on the persistency of short-rotation ryegrass tillers is shown by the results presented in Table 1.

TABLE 1. TILLER NUMBERS IN THE LAXLY GRAZED SHORT-ROTATION RYEGRASS PASTURE BEFORE AND AFTER DIFFERENTIAL TREATMENT ON 27 JANUARY 1961

Sampling date	Cutting treatment	No. of tillers per sq. ft.
25.1.61	Lax (3 in.)	523
	Hard (½ in.)	523
6.4.61	Lax (3 in.)	552
	Hard (½ in.)	72

A reduction of 86 per cent in tiller numbers per sq. ft. is shown in the hard-grazed pasture. Some of these losses were caused by the removal of growing points under intensive grazing, resulting in tiller death (Fig. 2). From observation some of the tiller death recorded was also due to other factors, such as tiller damage by the action of the Argentine stem weevil (*Hyperodes griseus* Hust.).

DISCUSSION

The two varieties of ryegrass studied are synthetic varieties, each based on seven unrelated parent plants. Perennial ryegrass is the earlier flowering variety, while short-rotation ryegrass is characterised by a relatively long period of flowering. In this respect it differs markedly from perennial ryegrass, which has a short period of flowering.

Although there is little published information available showing these characteristics of the two varieties, an indication of time of floral initiation and ear emergence in perennial ryegrass is obtained from results presented by Wilson (1959). For a selection of certified New Zealand pedigree strain material growing as ungrazed spaced plants 77 per cent of head emergence was shown to occur during a three-week period in October. Little emergence occurred after mid-November. For Italian ryegrass head emergence was more irregular. Most heads emerged between October and December, although some were still emerging as late as mid-February. Short-rotation ryegrass was not studied by Wilson (*loc. cit.*) but field observations indicate that head emergence and flowering behaviour of this variety are more similar to those of Italian ryegrass than to those of perennial ryegrass.

The results presented in Figs. 2 and 3 show that, for both ryegrass varieties, the highest percentages of tillers with elongated stems and emerged seed-heads were recorded on dates later than those reported by Wilson (*loc. cit.*) for ungrazed plants of perennial and Italian ryegrasses. Marked differences between grazing treatments are also

shown. The highest percentages estimated in the continuously-grazed perennial ryegrass pasture were recorded approximately one month later than those of the laxly-grazed pasture, whereas those of the continuously-grazed short-rotation ryegrass pasture were recorded approximately two months later than those of the corresponding laxly-grazed pastures.

These results suggest that the animals in the continuously-grazed pastures of both varieties removed a high proportion of elongating stems. In the perennial ryegrass pasture this occurred mainly during October and November, whereas in the short-rotation ryegrass pasture the effect was more pronounced and continued until mid-January because of the increased palatability of the elongating stems and seed-heads of this species. After these dates, it can be deduced that little or no removal occurred, as the percentages of elongated stems recorded in comparable treatments of each variety were similar and low.

Some of the differences recorded in Figs. 2 and 3 could also be the results of changes in the genotypic structure of the populations that had occurred between sowing and the beginning of this study. All pastures were autumn-sown in 1959. Following an establishment period of from 3–6 months, they were subjected to their different management treatments for a period of approximately 18 months. This included a period of reproductive growth in the preceding late spring and summer when stem elongation and seed-head emergence in the different pastures would have followed patterns similar to those recorded in Figs. 2 and 3. Under such conditions, differential tiller deaths through growing-point removal by grazing, as shown in Table 1, and differential plant renewal through reseeding, could markedly alter the genotypic structure of the populations. The occurrence of such changes in short-rotation ryegrass populations because of differences in pasture management has been shown in a previous study (Brougham *et al.* 1960).

The percentages and total numbers of vegetative tillers in the short-rotation ryegrass pastures were much lower than in the comparable perennial ryegrass pastures, particularly under lax grazing. For this reason, management systems applied to short-rotation ryegrass populations where a high proportion of the growing points of tillers is removed by grazing will result in a marked effect on persistency if little or no tiller regeneration follows. Such conditions occur during the drier and warmer summer months, as is shown by the results presented in Table 1 and in previous experiments (Brougham 1959, 1960). In these studies hard summer grazings, following either lax or intensive grazing, drastically reduced tiller densities.

However, it is suggested that short-rotation ryegrass can be maintained by intensively grazing such pastures before the onset of dry weather conditions in the late spring, followed by lax grazings over the drier summer months. This grazing system would result in an increased percentage of vegetative tillers because

of the removal of tillers with growing points above the 1 in. level during the intensive grazing (Fig. 2). Tiller regeneration due to increased light at the base of the plants following leaf removal at grazing (Mitchell and Coles 1955) would aso be encouraged.

In practice the production and persistence of short-rotation ryegrass shows wide variation because of the many different systems of management that are commonly applied to pastures containing the variety. The results of this and previous studies show that some of this variability can be attributed to its susceptibility to hard grazing during a relatively long period of reproductive activity over the late spring, summer, and early autumn. By employing grazing management systems which reduce the extent of reproductive activity, such as that outlined above, persistency of the variety can be maintained.

ACKNOWLEDGMENTS

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REFERENCES

Branson, F. A. 1953: Two new factors affecting resistance of grasses to grazing. *J. Range Mgmt. 6:* 165–71.

Brougham, R .W. 1959: The effects of frequency and intensity of grazing on the productivity of a pasture of short-rotation ryegrass and red and white clover. *N.Z. J. agric. Res. 2:* 1232–48.

————1960: The effects of frequent hard grazings at different times of the year on the productivity and species yields of a grass-clover pasture. *Ibid. 3:* 125–36.

Brougham, R. W.; Glenday, A. C.; Fejer, S. O. 1960: The effects of frequency and intensity of grazing on the genotypic structure of a ryegrass population. *Ibid. 3:* 442–53.

Mitchell, K. J.; Coles, S. T. J. 1955: Effects of defoliation and shading on short-rotation ryegrass. *N.Z. J. Sci. Tech. A36:* 586–604.

Wilson, J. R. 1959: The influence of time of tiller origin and nitrogen level on the floral initiation and ear emergence of four pasture grasses. *N.Z. J. agric. Res. 2:* 915–32.

STAPLE STRENGTH

III. Variations through the wool selling season

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ABSTRACT

There is a marked decrease in the staple strength of wool offered for sale at both Christchurch and Dunedin as the selling season progresses. The staple strength of wool sold in the pre-Christmas sales is twice that of wool sold after Christmas. This decrease, which is more marked in the Crossbred than in the fine wools, may be assessed by hand testing staples for soundness, and can thus be measured by the wool trade without the need of any special testing equipment.

During the present investigations, as the selling season progressed, the break level rose to 40 per cent; the proportion of staples which could be hand broken reached about 75 per cent, while the amount of cotted wool rose to about 20 per cent. Over a period of 5 seasons, the unsound poor style wool sold at the last sale brought a higher price than the sound better syle wool in the first sale.

To obtain sound good style wool, sheep should be shorn either before lambing time or as soon as practicable after lambing.

INTRODUCTION

It is generally accepted by both wool brokers and wool buyers that as the selling season progresses the general style of the wool offered for sale deteriorates, and that the proportion of unsound fleece wool increases. This paper records the results of a series of investigations into the staple strength of the wool offered for sale at Dunedin over a period of 5 years and at Christchurch during one year.

While a break may occur at any time of the year, this paper is concerned with the break or unsoundness which frequently occurs in the late winter/early spring period about the time of lambing. This region of low tensile strength in the staple is variously described as the winter-fine, winter-break, or lambing-break region. The term lambing-break refers only to the time of occurrence and not to the cause of the break, as dry ewes, hoggets, wethers, and rams may also develop a break at this time of the year.

MATERIALS AND METHODS

Selection of samples

In any wool survey the amount of wool in the samples examined is only a very small proportion of the wool available, so that care is necessary both in sampling and in the interpretation of the results. For this study of changes in staple strength over the various sales of each wool selling season, the principal samples were obtained from selected bins at brokers' stores which would be available at each of the 4 main sales. Bin wool is relatively constant in count (Bradford count or quality number) and style, and, being derived from numerous farms, is more representative of the districts from which it is obtained than wool from individual farms which is shorn at the one time and sold at one sale.

Wool stores frequently have 3 or 4 bins of different style fleece wool for each count grade. These might be graded, for example, AAA MXBd, AA MXBd, A MXBd, and MXBd. The stores aim at keeping the count and style of the wool in these bins constant throughout the season. As the general style of the wool coming into the store deteriorates with the progress of the season, the store may not be able to continue to make its top bin, e.g., AAA MXBd, and wool which would have been graded as A MXBd or MXBd early in the season may be upgraded to AA MXBd and A MXBd respectively at later sales to ensure a sufficient quantity of wool in these bins to make a main sale lot. Bins selected for sampling were the best bins of the particular count which would be made right through the selling season.

1. Samples were taken from one Dunedin woolstore through 5 successive seasons, 1956–7 to 1960–1, from the following 4 fleece wool bins: 56/58s Halfbred (HB); 50/52s fine Crossbred (FX); 48/50s medium Crossbred (MX); and 46/48s strong Crossbred (SX).

2. In view of the results of the 1956–7 samples it was decided to sample at 3 more stores in Dunedin and 3 in Christchurch in 1957–8 to obtain a more representative sample of the wool sold in these two centres. Samples were taken from bins similar to those in item 1, and also from a 60/64s Merino (M) bin.

3. An intensive study was made on 1 bale of medium Crossbred wool purchased from one bin at the first Dunedin wool store at each sale in the 1958–9 season. A similar study was carried out in the 1959–60 season on 2 bales from each sale of a fine Halfbred bin.

4. In the 1958–9 season one sample was taken from every open bale of farmers' and bin wool which was classified in the catalogue of the first Dunedin woolstore as XBd.

Sampling procedure

When the wool was open for display before a sale, 20 samples were taken from each of the selected bins, one or more samples being taken from each bale, the precise number depending upon the number of bales which were open for inspection. Where there were only 3 or

4 open bales in the sale lot, particularly in the case of the Merino bins, a sampling hook was used to obtain samples from different parts of the bales.

Testing procedure

Samples were conditioned at 68°F and 65 per cent r.h. for at least 24 hours prior to testing.

Staple strength. One staple from each of the 20 samples per bin lot was tested for staple strength using the method given by Ross (1960a). The test lengths used were: Merino—1.0 in.; Halfbred—1.5 in.; Crossbred—2.5 in. The butt 0.75 in. of the staple was clamped in the lower jaw, so that usually the "break level" was between the jaws, and the minimum strength of the staple was measured.

Hand breaking grade. The strength of one staple per sample which had first been measured for staple length was assessed by hand breaking. Staples were graded into one of five grades (Ross *loc. cit.*): Grade 5, unbreakable; Grade 4, very slight break—the staples "gave" on stretching but did not break completely; Grade 3, slight break—the staples gave under strong tension; Grade 2, break; Grade 1, bad break.

Break level. The distance was measured from the staple butt to the level of the break in staples of grades 1, 2, and 3 which broke into two portions. The break level was then expressed as a percentage of the staple length up from the butt. This distance was rather higher than in the intact staple, owing to fibre straightening during breaking. Where fibres broke at two distinct levels in the staple, the lower level was taken as the break level.

Other measurements. Data were also obtained on the crimp, diameter, and yield of each bin. These results, which show the inter-relation of the various sample characteristics, will be discussed in a later publication.

Sale dates and shearing times

The Dunedin sale dates for the 1956–7 to 1960–1 seasons and the approximate catalogue closing dates, which indicate when the wool was shorn, were as follows:

1st sale: 15–24 October. The closing date for this sale was early October at first but with the increase in the amount of pre-lamb shorn wool in later years the closing date became towards the end of September.

2nd sale: 10–15 December. The closing date, which in 1956-7 was the end of November, became later very early in November.

3rd sale: 23 February–6 March. Closing date mid-January.

4th sale: 23 April–6 May. Wool was accepted almost up to the sale date.

Sale and closing dates for the Christchurch woolstores sampled in 1957–8 were:

1st sale: 30 October. Closing date: mid-October.

2nd sale: 4 December. Closing date: mid-November.

3rd sale: 12–14 February. Closing date: Christmas.

4th sale: 21 April. Closing date: just before sale.

RESULTS

1. *Five seasons' samples from one Dunedin woolstore*

The general characteristics of the 4 bins sampled at each sale for the 5 years are given in Table 1.

TABLE 1. MEAN CHARACTERISTICS OF FOUR BINS SAMPLED AT EACH SALE FOR THE SEASONS 1956–7 TO 1960–1

		Halfbred	Fine Crossbred	Medium Crossbred	Strong Crossbred
Count		56/58s	50/52s	48/50s	46/48s
Staple length	(in.)	3.9	5.3	5.9	5.9
Crimps/inch		7.0	3.8	3.3	3.1
Fibre diameter	(μ)	28.7	35.2	36.3	37.1
Fibre diameter of length tested	(μ)	29.2	34.8	35.2	36.2

The mean values for the different characteristics were of the expected order for the different bins, although there was no difference in length betwen MX and SX bins, and comparatively little change in diameter between the 3 Crossbred bins, although there was an appreciable change in crimp frequency. The fibre diameters of the sections of the Crossbred staples tested for staple strength were only slightly lower than the diameters of the complete samples.

The mean staple strength values of the 20 staples per bin are given in Table 2.

The following observations were made on these results:

(a) For all bins there was a decrease in the mean staple strength as the selling season progressed.

(b) The decrease was less pronounced in the Halfbred than in the Crossbred bins.

(c) The strengths of sound Crossbred wools, irrespective of count, were similar, as would be expected from the method of testing, so that the strengths of the 3 Crossbred bins may reasonably be combined and considered as one group. The means of the combined results are also shown in Table 2.

TABLE 2. THE STAPLE STRENGTH (lb/g) AT EACH SALE FOR FIVE SEASONS OF A HALFBRED, AND A FINE, MEDIUM, AND STRONG CROSSBRED BIN FROM ONE DUNEDIN WOOLSTORE

(Test length: Halfbred—1.5 in., Crossbred—2.5 in.)

Bin	Season	Sale				
		1st	2nd	3rd	4th	Mean
HB	56–57	197	211	190	127	181
	57–58	188	216	150	157	178
	58–59	251	215	218	182	217
	59–60	254	185	222	167	207
	60–61	245	210	126	129	178
	Mean	227	207	181	152	192
FX	56–57	142	102	66	49	90
	57–58	150	105	58	38	88
	58–59	173	155	69	64	115
	59–60	144	90	76	75	96
	60–61	124	88	70	73	89
	Mean	147	108	68	60	96
MX	56–57	141	110	69	66	97
	57–58	152	124	67	60	101
	58–59	146	139	72	93	113
	59–60	152	72	76	75	94
	60–61	150	95	56	72	93
	Mean	148	108	68	73	99
SX	56–57	133	132	70	45	95
	57–58	146	121	66	78	103
	58–59	143	168	79	82	118
	59–60	142	86	73	88	97
	60–61	172	104	75	72	106
	Mean	147	122	73	73	104
Mean Crossbred		147	113	70	69	100

(d) The general level of strength of the wools was very similar in 4 of the 5 years, while it was higher in the 1958–9 season, in which the strength of the second sale samples was similar to that of those of the first sale. The soundest Crossbred bin had a trength of 168 and the weakest 38 lb/g.

(e) The staple strength of wool in the third sale was similar to that in the fourth.

Table 3 shows data on the variability of staple strength within the 3 Crossbred bins sampled in the 1956–7 season.

TABLE 3. THE VARIATION IN STAPLE STRENGTH WITHIN THE 3 CROSSBRED BINS SAMPLED IN 1956–7

		Sale			
		1st	2nd	3rd	4th
Staple strength	(lb/g)	139	115	68	53
standard deviation	(lb/g)	46	48	35	28
coefficient of variation	(%)	33	42	52	53
Five soundest staples	(lb/g)	216	188	141	117
Five weakest staples	(lb/g)	64	40	18	18
Range	(lb/g)	152	148	123	99
Work to break	(cm lb/g)	543	457	303	233

Observations on the results were:

(a) Within each sale there was a wide variation in staple strength. As measured by the coefficient of variation the variability increased from 33 per cent at the first sale to 53 per cent at the last.

(b) As the variation in staple strength within a group was large, the staple strength of the 5 weakest staples in the first sale was only half that of the 5 soundest staples in the last sale.

(c) The mean first sale strength of 139 lb/g was low compared with the strength of the 5 strongest staples, 216 lb/g, which indicates the approximate upper limit of staple strength for wools of this type at a 2.5 in. test length. The 5 weakest staples in the last 2 sales, with a strength of only 18 lb/g, thus had only 8 per cent of the strength of the soundest staples.

Also shown in Table 3 are the mean values for the total amount of work required to break these staples (Ross 1960a). These work values bear a comparatively constant ratio to the corresponding mean staple strength values. Thus staple strength measurements, which are based on the maximum load sustained by the staple, give a close estimate of the total work required completely to break the staples. While the total work values may be more easily interpreted, they are much more laborious to measure.

Full data on hand-breaking grade, the percentage of staples which could be hand broken (Grades 1, 2, and 3), and the break level were not obtained from the one Dunedin woolstore in the 1956-7 season. Table 4 shows the mean values for these data for the 4 subsequent years.

Hand-breaking grade and staple strength showed a similar decline through the selling season, while the break level and the percentage of staples which could be hand broken both increased through the season.

TABLE 4. MEAN VALUES FOR HAND BREAKING GRADE, PERCENTAGE OF STAPLES OF GRADES 1, 2, AND 3, AND BREAK LEVEL FOR THE HALFBRED AND THE COMBINED CROSSBRED BINS

	Bin	Sale			
		1st	2nd	3rd	4th
Hand breaking grade	HB	4.4	3.8	2.7	2.4
	XB	4.7	3.6	2.3	2.5
Percentage staples grades 1, 2, 3	HB	19	34	68	74
	XB	6	44	78	72
Break level %	HB	—	24	28	40
	XB	—	25	32	39

2. Five bins sampled at 4 Dunedin and 3 Christchurch woolstores in 1957–8

The average counts of the bins sampled at Christchurch and Dunedin, as assessed by members of the New Zealand Wool Commission, were as follows:

Strong Crossbred	46s part 48s
Medium Crossbred	48/50s
Fine Crossbred	50s part 52s
Halfbred	58s part 60s
Merino	64s part 60s

The average style gradings of the 5 bins at each of the 4 sales (where BB style = 2 and B style = 1) were as follows:

	Sale			
	1st	2nd	3rd	4th
Dunedin	1.6	1.5	1.4	1.0
Christchurch	1.2	1.4	1.1	1.1

The Dunedin wool was of better style than that sampled at Christchurch, while at both centres the wool sold at the later sales was of poorer style than that sold earlier in the season. In general the style of the finer bins was better than that of the Crossbred bins.

Table 5 shows the principal strength data for the averages of the 4 Dunedin and the 3 Christchurch woolstores. The following observations were made on these results:

(a) The general level of staple strength at the two selling centres was similar.

TABLE 5. STRENGTH DATA ON BINS FROM 4 DUNEDIN AND 3 CHRISTCHURCH WOOLSTORES, 1957–8

	Bin	Dunedin				Christchurch			
		Sale				Sale			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th
Staple* strength lb/g	M	283	290	262	283	297	219	278	263
	HB	174	181	194	128	182	187	105	168
	FX	132	91	67	55	143	94	83	112
	MX	146	105	73	55	130	110	93	78
	SX	150	114	80	89	124	116	91	102
Hand breaking grade	M	3.4	3.6	2.6	2.7	4.1	3.3	2.9	2.9
	HB	3.9	3.4	2.2	2.1	3.4	3.2	2.6	2.5
	FX	4.4	2.9	2.4	2.0	3.7	3.1	3.0	2.4
	MX	4.5	3.0	2.3	2.3	3.8	3.6	3.5	2.8
	SX	4.7	3.7	2.7	3.2	4.5	4.2	2.8	3.1
Percentage grades 1, 2, and 3	M	40	49	81	66	33	55	70	68
	HB	35	50	79	81	53	53	67	67
	FX	18	66	79	86	38	57	62	80
	MX	16	63	75	81	33	42	47	68
	SX	10	40	70	55	17	23	62	60
Break level %	M	36	28	35	37	52	38	42	42
	HB	29	27	31	41	40	35	35	38
	FX	25	21	31	43	49	27	34	38
	MX	31	25	31	39	38	28	37	39
	SX	30	22	33	38	34	35	33	42

* Test lengths: M = 1.0; HB = 1.5; F, M, and SX = 2.5 in.

(b) The decrease in staple strength through the season occurred at both centres, and was most marked in the Crossbred bins, there being little change in the strength of the Merino bins throughout the season. Similar trends were shown by the hand breaking grade results.

(c) About 60 per cent of the total Merino, Halfbred, and fine Crossbred staples fell into the poorer hand breaking grades 1, 2, and 3, together with 54 per cent and 42 per cent in the medium and strong Crossbred bins.

(d) The break level was high in the Merino wools, being higher for the Christchurch than for the Dunedin samples. The break level was very similar for the 3 types of Crossbred wool at each of the 3 later sales and rose as the selling season progressed.

3. Staple strength of two bales of fine Halfbred and one of medium Crossbred wool bought from the same bins at each sale

Three staples from each Halfbred and 4 staples from each Crossbred fleece were tested for staple strength, the results being given in Table 6.

TABLE 6. DATA ON TWO BALES OF FINE HALFBRED AND ONE OF MEDIUM CROSSBRED WOOL

	Fine Halfbred				Medium Crossbred			
	Sale				Sale			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
No. of fleeces	79	82	79	82	40	36	34	40
Staple length (in.)	3.5	3.5	3.5	3.5	5.2	6.0	6.0	6.1
Crimps/inch	9.5	9.1	10.0	9.8	3.4	3.8	3.8	3.8
Staple strength (lb/g*)	153	142	125	95	120	66	67	95
Hand breaking grade	3.3	3.3	2.9	2.2	4.4	2.0	2.2	2.9
% staples grades 1, 2, 3	52	52	60	66	15	92	85	75
Break level %	21	21	19	40	13	26	26	36

* Test length: Halfbred—1.6 in. (4 cm), Crossbred—2.5 in.

The Halfbred bin was very consistent in length and crimp throughout the season, and when the 8 bales were viewed together at the end of the season they all appeared to be of good BB style. The break in the wool from the 4th sale was not apparent to the eye but it was noticeable to wool classers on hand testing for soundness. The general level of strength in this bin was low compared with the Crossbred wool when differences in test length were allowed for. The proportion of staples of hand breaking grades 1, 2, and 3 was high at all the sales, although only in the last sale was the break level high up the staple. In the Crossbred wool the break level was also high in the fourth sale, but the weakest wool was in the second and third sales.

4. The staple strength of farmers' and bin wool, 1958–9

The staple strength, which was similar at the first two sales, decreased markedly in the third and fourth sales. This was to be expected from the results given for the combined Crossbred bins at this store for the 1958-9 season (Table 2). The mean strength of the Crossbred bin lines was 154 lb/g in both the first and second sales, so that the poorer style wool included in the farmers' and the other XBd

TABLE 7. STAPLE STRENGTH OF FARMERS' AND BIN LOTS OF XBD WOOL (46/48s) FROM A DUNEDIN WOOLSTORE IN THE 1958–9 SEASON

	Sale			
	1st	2nd	3rd	4th
No. of staples	273	67	426	240
Staple length (in.)	5.7	6.0	5.4	6.3
Crimps/inch	3.2	3.1	3.2	2.9
Staple strength (lb/g/2.5 in.)	133	134	99	63

bins resulted in a decrease in the mean strength of 20 lb/g. The results found for the bin wool are therefore applicable to the other wool in the sales, although the level of strength in the bins tested was higher than the general level.

The variation in strength of this wool was so large that a corresponding variation would be expected in the quantities of wool catalogued by the woolstore into its own style groups. Table 8 shows the store classification of this XBd fleece wool and the percentage of the total fleece wool (excluding hogget and lambs' wool) classified as cotts.

TABLE 8. WOOLSTORE STYLE CLASSIFICATION OF XBD BALES (EXCLUDING HOGGET AND LAMBS WOOL), 1958–9

		Sale			
		1st	2nd	3rd	4th
AA XBd	%	20	19	12	13
A XBd	%	61	61	48	18
XBd	%	19	20	40	69
Cotts	%	0.2	0.5	13.1	21.6

The style distribution in the first and second sales was very similar, with about 20 per cent each of the best and poorest style wools. The percentages of both the better style AA XBd and the average A XBd wool decreased in the later sales, while the percentage of poor XBd wool increased from 40 per cent at the third sale to 69 per cent at the fourth sale.

5. Style and price

The style of the Halfbred wool sold at the one Dunedin woolstore over the 5 seasons based on the style grading classification of the New

Zealand Wool Commission was BB for the first, second, and third sales and BB/B for the fourth sale. For the Crossbred bins the wool was of BB style for the first and second sales, BB/B for the third sale, and only B for the fourth sale.

Fig. 1 shows the mean staple strength values for the Halfbred and the 3 Crossbred bins, together with the average greasy and clean prices paid at auction for them.

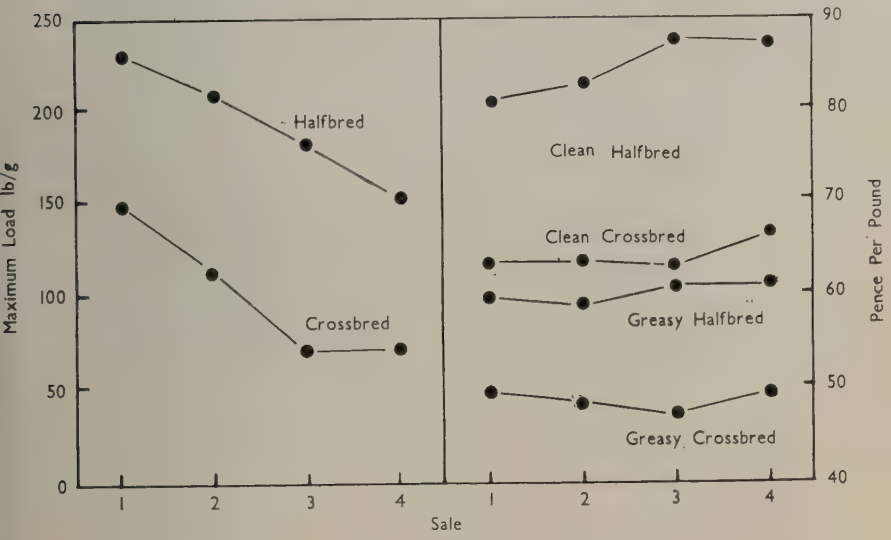

Fig. 1.—The staple strength of Halfbred wool (lb/g/1.5 in.) and Crossbred wool (lb/g/2.5 in.), together with the average greasy and clean price received at each sale for the seasons 1956–7 to 1960–1.

DISCUSSION

The results show a relatively consistent pattern, both between years and selling centres, in the seasonal changes in the staple strength of the Crossbred wool offered at auction. In relation to the time of shearing, the pre-lamb shorn wool sold at the first sale was on the average twice as sound as the wool sold at the last two sales, which was shorn later than the end of November. The wool sold at the second sale, which would have been shorn before the end of November, was of intermediate strength.

Apart from the different times of shearing, the wool in the different sales is composed of different proportions of wool from different classes of stock and types of country. The first sale wool, which is almost completely pre-lamb shorn, is sound, as the fibres taper to both ends, thus avoiding the "winter-fine" waist region (Story and Ross 1960). However, as may be seen from Table 3, there is still considerable scope for improvement in the staple strength of some of this wool.

There is also a proportion of pre-lamb shorn wool in the second sale, together with a high proportion of wool from dry ewes, hoggets, wethers, and rams. In the later sales there is a high proportion of wool from wet ewes where shearing has been delayed until lamb weaning. The similar strength of the third and fourth sale Crossbred wools (Table 2) indicates that no stress of later origin than the winter break period was of importance. The average value of 100 lb/g for a Crossbred wool is a useful guide for assessing soundness.

This decrease in strength cannot be accounted for in terms of changes in the other characteristics of the bins as the selling season progresses. Staple length and crimps per inch were relatively constant (Table 1), while the mean airflow fibre diameter of the wool cut from the clamps was only slightly and consistently lower than the diameter of the complete bin sample. It is of interest to note that the probable value of 3.2 crimps per inch (Table 1) for 48s count wool is the same as that found by Ross (1959), while the corresponding fibre diameter is about half a micron finer.

The fact that the Crossbred hand breaking grade results (Tables 4, 5, and 6) show a pattern similar to the staple strength results indicates that these changes in staple strength may be assessed by the wool trade without the need of any specialised testing equipment. This will not be done, however, until there is a clearer appreciation of the importance of soundness during manufacture. At the present time wool must be of very low staple strength before it is classified by the wool trade as tender; this is in part due to the usual method of assessing soundness employed by the trade—flicking the taut staple. By this method only some grade 1 and a few grade 2 staples would be classified as unsound. The tip and butt of the staple must be firmly held and strong tension applied adequately to grade staples for soundness.

The decrease in staple strength and hand-breaking grade was by no means as pronounced through the season in the Halfbred and Merino wool as it was in the Crossbred wool. The decrease in hand breaking grade for the 1957–8 season (Table 5), however, showed a greater seasonal decrease than the staple strength values would indicate. For the 5 seasons over which the Halfbred bin was sampled (Tables 2 and 3) there was an appreciable decrease in strength and grade through the season.

The smaller decline in strength in these fine wools may be due to several factors. As discussed later, the general level of strength of the Halfbred and Merino wool is similar to but appreciably lower than, that of the Crossbred wool, which may partly account for the smaller range of strengths of the fine wool. Because of the smaller staple and test lengths of the fine wools, and the variability of the break level, the region of lowest tensile strength may have been held within the clamps more frequently for these wools, which would result in high staple strength values. The seasonal fibre diameter rhythm may be less pronounced in the fine wools, while stress factors may not be so coincident with the period of minimum fibre diameter as with the Crossbred sheep.

The relation between staple strength and hand breaking grade for the 2,800 Merino, Halfbred, and Crossbred staples (Table 5) is shown in Fig. 2.

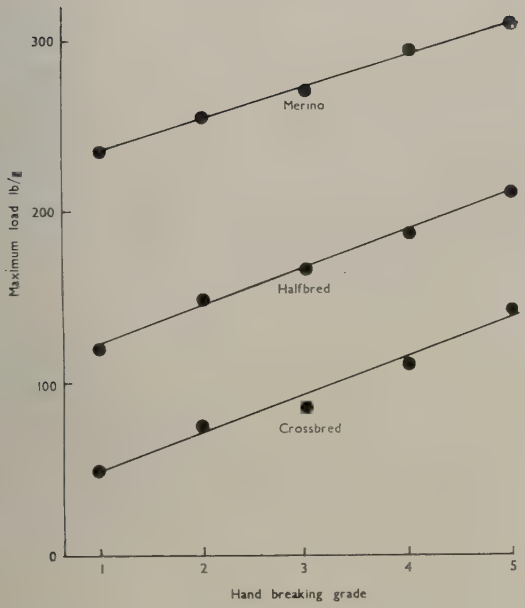

Fig. 2.—Relation between hand breaking grade and the staple strength of Merino (lb/g/1.0 in.), Halfbred (lb/g/1.5 in.), and Crossbred (lb/g/2.5 in.) wool.

For each type of wool there was a linear relation between hand breaking grade and mean staple strength. It may be seen that the decrease in strength in lb/g from Grade 5 to Grade 1 was similar for each type of wool, so that proportionately it was much greater for the Crossbred wool. Of the Crossbred staples 18 per cent were of Grade 1, and of these only 32 per cent had actual breaking loads of 7 lb or less, while 48 per cent had breaking loads of from 7 to 15 lb. Based on the traditional strength of 7 lb for a combing wool, it may be seen that over 90 per cent of the Crossbred wool would be classified by the wool trade as sound. With an average staple strength of 100 lb/g (Table 2) and the clean weight of the 2.5 in. test length of 0.3 g then an average Crossbred staple would have a breaking strength of 30 lb. This value is equivalent to a strength midway between the Grade 3 and 4 samples, and is much higher than the 10–11 lb which Roberts *et al.* (1960) found was the maximum tension exertable without undue strain by the average man. Both the longer staple length of the Crossbred wool and the method of testing for staple strength would account for this difference.

As it is desirable to use the longest possible test length, different test lengths were used for the 3 types of wool. Because of this, care must be taken in comparing the strengths of the different types of wool, particularly if they are unsound (Ross 1961). Allowing for differences in test length, the strengths of the Merino and Halfbred

wools were almost identical (see Tables 2 and 4, also the grade 5 staples of Fig. 2), but they were from 10–25 per cent weaker than the Crossbred staples. The fine wool samples were even weaker in relation to the Crossbred wools if allowance is made for the fact that even in bundles of similar fibres strength decreases with increasing test length (Ross 1960b).

The break level of 20–25 per cent at the second sale increased to 33 per cent at the third sale and 40 per cent at the final sale. The wool trade, however, takes little account of the break level even though this is a very serious fault in worsted processing (Ross *et al.* 1960). As the tension in a fibre during carding increases towards its centre, fibre breakage will be higher in a wool with a high break level than in a wool of the same staple strength which has a low break level.

A comparatively high proportion of the Christchurch first sale wool (Table 5) could be hand broken, and the average break level was high in the staple. Some of these staples had tip breaks, that is, they were shorn in the late autumn or early winter and developed a winter-fine region just below the tip. From the point of view of the manufacturing performance of the wool, a break level of 80 per cent would be approximately equivalent to a break level of 20 per cent.

The proportion of staples which could be hand broken (Tables 4, 5, and 6) increased to a surprisingly high level of about 75 per cent in the later sales. This was not the result of selection of thin staples for testing but of the high tensions which were applied to the staples.

Cotted wool is usually classified, both mentally and in catalogue listings, separately from unsound wool. The fact that cotting is an obvious manifestation of a break, thus allowing the free fibre ends to cott, is frequently overlooked. Any reduction in the incidence of break will therefore automatically reduce the amount of cotted fleece wool. The longer the period from the formation of the break to the time of shearing, the longer the time for the free fibre ends to move in the fleece and form a cott and the greater the proportion of "hard" cotts which will be formed.

It is apparent from the foregoing discussion that there is a marked deterioration in the quality of the wool in a particular bin as the selling season progresses. One need only consider, for example, the results of the Dunedin wool store which was sampled over a period of 5 years. The wool in the bins in the last sale was of low staple strength and of poor style, the break level was high, and a large proportion of the staples could be hand broken, yet the average price paid for this wool was higher than the price paid for the better style sound wool sold in the first sale. There would appear to be little if any relation between the average quality of the wool in a sale and its average selling price. While within a sale a small premium may be paid for wools of a better style, this does not necessarily apply in a comparison of prices and styles between several sales. The lack of a clear financial premium for sound better-style wool is probably the greatest deterrent to the improvement of the standard of the New Zealand wool clip.

There is a marked seasonal rhythm in the wool growth of Romney ewes, the fibre diameter being markedly reduced in the late winter/early spring period (Story and Ross 1960). Superimposed upon this seasonal rhythm are the stresses of inadequate nutrition, disease, pregnancy, lactation, and other factors, which may cause a further reduction in fibre diameter and/or fibre shedding. This will result in the formation of a break, that is, a region of low tensile strength. Few data are available on the relative importance of these stress factors in the formation of a break; no doubt nutrition plays a predominant role. Ross and Lewis (1958) found little difference between the severity of break in dry, single- and twin-bearing ewes, while Ross (1961) found that staple strength varied markedly over the body of each individual ewe.

Until more is known of the causes and methods of avoiding a break, the most obvious recommendation which can be made to overcome the decrease in strength and style and the increase in the break level of the later shorn wool, is to shear the ewes as early as practicable. Where pre-lamb shearing is not practical, the sheep should be shorn as soon as possible after lambing time. While this survey has been confined to South Island wools, it is probable that a similar situation occurs in the North Island, where there is also a decrease in the general style of the wool as the selling season progresses, particularly in the final sales. The absolute drop in staple strength may not be so marked in the North Island, however, as pre-lamb shearing—leading to high staple strengths—is largely confined to the South Island. The incidence and degree of break in the North Island wools will also be affected by the increasing practice of early or twice yearly shearing.

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REFERENCES

Roberts, N. F.; James, J. F. P.; Burgmann, V. D. 1960: *J. Text. Inst. 51:* T935.

Ross, D. A. 1959: *N.Z. J. agric. Res. 2:* 214.

————1960a: *Ibid. 3:* 503.

— ————1960b: *J. Text. Inst. 51:* T240.

————1961: *N.Z. J. agric. Res. 4:* 57.

Ross, D. A.; Lewis, K. H. C. 1958: *Proc. N.Z. Soc. Anim. Prod. 18:* 141.

Ross, D. A.; Bratt, R. L.; Story, L. F. 1960: *J. Text. Inst. 51:* T907.

Story, L. F.; Ross, D. A. 1960: *N.Z. J. agric. Res. 3:* 113.

SEASONAL VARIATION IN SPORE NUMBERS OF *Pithomyces chartarum* IN 1960 AND 1961 IN THE WAIKATO

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ABSTRACT

In 1960 and 1961 surveys were made on grassland herbage of paddocks in the Waikato region of New Zealand* with a mobile volumetric spore trap to determine the nature and magnitude of the fluctuations in estimated spore numbers of *P. chartarum*, the fungus implicated in facial eczema disease of sheep and cattle. The relation between these fluctuations and the weather conditions as recorded at a reference centre was examined.

The trends in spore counts on the herbage of reference paddocks can give reliable indications of the trends in counts on paddocks in the surrounding farming district. The count levels at any one time on different paddocks may vary considerably.

Trends in estimated spore trap counts are markedly influenced by weather conditions. Major peak counts occurred in dry weather following moist warm conditions.

Although estimated spore trap counts do not directly indicate spore load on grazed herbage, it is considered that the presence of a danger period for grazing animals is indicated when a rapid upward trend in spore counts occurs following a moist warm period.

INTRODUCTION

Pithomyces chartarum (Berk. & Curt.) M.B. Ellis is implicated in the intoxication of sheep** suffering from facial eczema (Percival 1959). Spores of the fungus contain the toxin sporidesmin (Synge and White 1959; Clare in press), and they may occur in large numbers on the green leafage ingested by grazing animals. A relation has been established between relatively high counts of spores and the occurrence of facial eczema disease in lambs (Thornton and Sinclair 1960). The grass sampling, washing, and haemocytometer count procedure adopted by Thornton and Sinclair, while precise, is time-consuming, and in its present form is not adaptable to the rapid collection of data. Smith *et al.* (in press) found that there was a broad general relation between estimated spore counts on herbage obtained with a mobile volumetric

* The basin of the Waikato and Waipa rivers in the Auckland Province.

** The disease also occurs in cattle.

spore trap (Brook 1959) and the severity of facial eczema in lambs in controlled grazing experiments. The mobile spore trap and slide count procedure provides a rapid means of assessing the numbers of spores which can be detached from herbage.

The incidence of facial eczema varies from season to season, and its severity appears to be related to weather conditions (Mitchell *et al.* 1959) and litter (substrate) formation (Thornton and Sinclair 1960; P. J. Brook pers. comm.).

This paper reports the results of surveys of pasture herbage made in the Waikato in 1960 and 1961 to determine:—

(1) The nature and magnitude of the fluctuations in the estimated spore count as obtained with the mobile spore trap on grazed paddocks throughout the area.

(2) The relation between these fluctuations and the weather conditions as recorded at the reference point—Ruakura Animal Research Station.

In 1961 the Ruakura survey was integrated with a national survey. This latter was undertaken by the Department of Agriculture to determine whether spore trap counts could provide additional information to assist local Facial Eczema Warning Committees which provide warnings (largely based on local agricultural experience and the occurrence of moist warm weather) to graziers (Anon. 1961).

EXPERIMENTAL

1960 SURVEY

Methods

Spore trap and counting techniques

Spore trapping was done with a standard Brook trap on herbage of 283 paddocks of 8 sheep farms, selected for their proneness to facial eczema at Morrinsville, Maungatautari, Ngaruawhia, Waharoa, Te Awamutu, Tuhikaramea, and Hamilton. A total of 1,800 slides was obtained from these farms, and a further 2,150 slides were taken in 22 paddocks on Ruakura Sheep Farm. The spore trap was operated until 1 cu. ft. of air had passed through it. Assessments of spore numbers were made microscopically using a ×8 ocular and ×43 objective. A disc with a square aperture of 8 mm side fitted in the ocular gave a counting field of 0.29 mm side. A continuous strip was counted down the centre of the 14 × 2 mm spore deposit on the trap slide, and the number of spores counted multiplied by 7 gave an assessment of the number of spores collected from each cu. ft. of air passing through the trap.

Results

Figs. 1, 2 and 3a summarise the results obtained in 1960. The weather data given in Fig. 3 are those collected at Ruakura. In

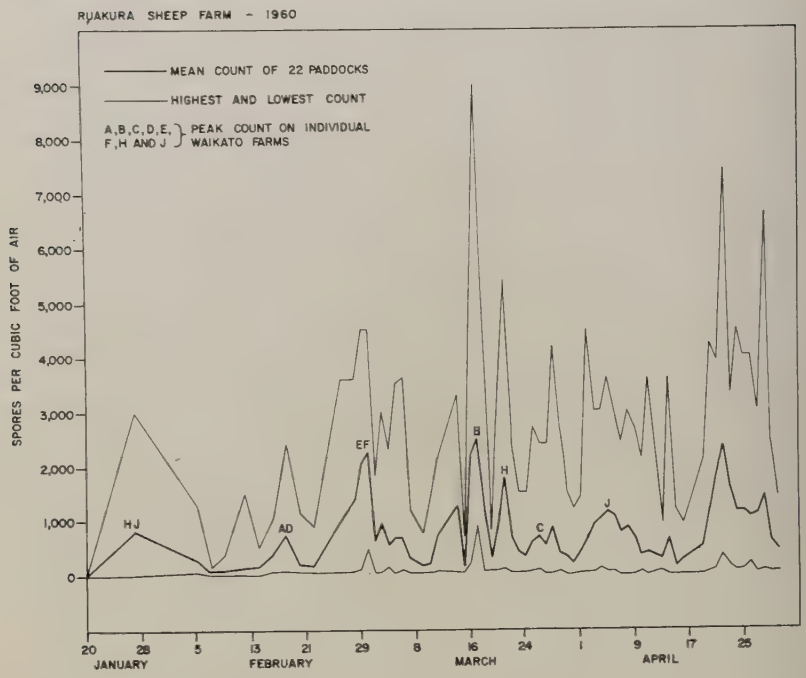


Fig. 1.—Spore trap counts of *P. chartarum* on paddocks of Ruakura Sheep Farm, 1960.

considering the results it is necessary to remember that weather data* given for a particular date refer to the preceding 24 hours and that spore count data are those of the current date. Until the end of March the weather was very wet; this affected the spore-trapping programme, and may explain in part why spore counts in 1960 were generally low. Although the spore count on one paddock on Ruakura Sheep Farm was 9,000 in mid-March (Fig. 1), the mean figure on 22 paddocks was approximately 2,500 spores per cu. ft. of air at that time. In Fig. 2 the counts on individual paddocks at Ruakura Sheep Farm are compared. The trends in spore counts on these paddocks agree well.

Peak counts recorded on Ruakura Sheep Farm coincided with the maximum count noted on paddocks on outside farms in the Waikato (Fig. 1). The highest count on each outside farm did not necessarily coincide with the highest count at Ruakura but this might have resulted from insufficient data. Paddocks with extreme counts on Ruakura (Figs. 1 and 2) showed the same trends in spore numbers as the mean of 22 paddocks.

* Weather data are based on 9 a.m. readings of instruments.

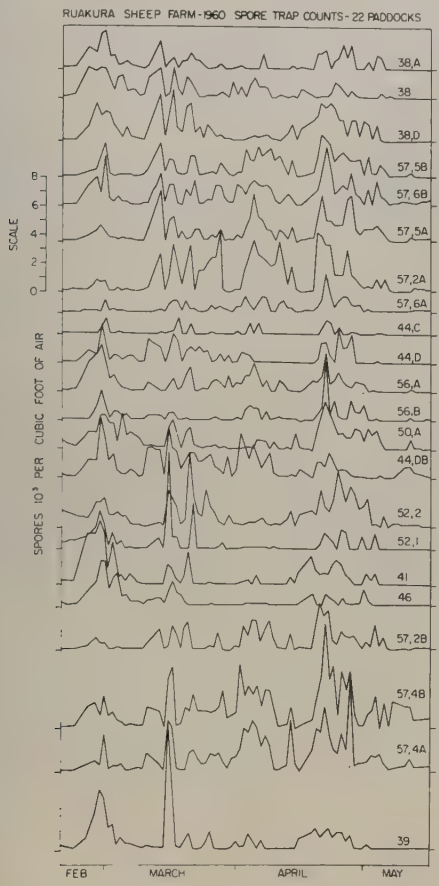
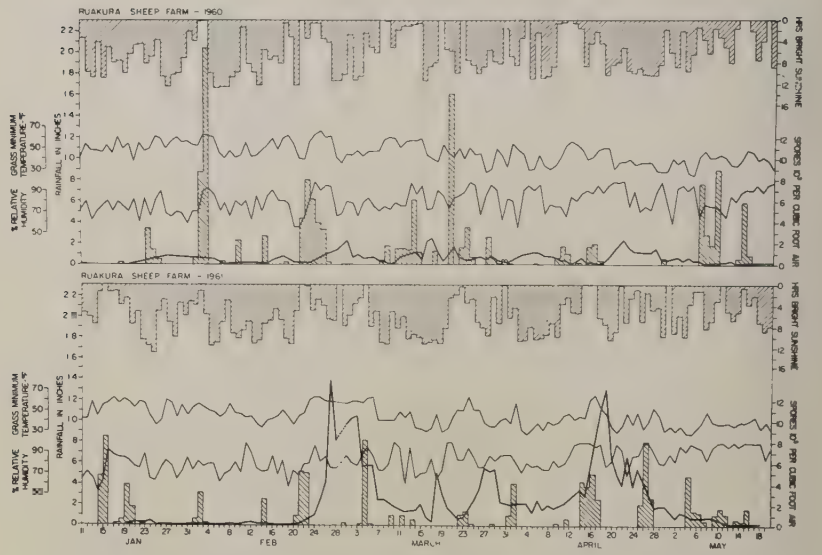


Fig. 2.—Spore trap counts of *P. chartarum* on individual paddocks of Ruakura Sheep Farm, 1960.

In two cases in 1960 (Fig. 3a) it seemed possible to establish a general relation between a period of wet weather, a grass minimum temperature for a substantial period of over 55°F, and a subsequent increase (after a lag phase) in spore numbers as in the table.

WEATHER AND SPORE COUNT PEAKS 1960

Period of rainfall	Rainfall in inches	Period of grass minimum temp. above 55°F.	Date of subsequent spore count peak	Peak count spores per cu. ft. of air
Feb. 21–27	2.79	Feb. 23–27	Mar. 1	2,250
Mar. 10–15	1.17	Mar. 12–16	Mar. 17	2,500



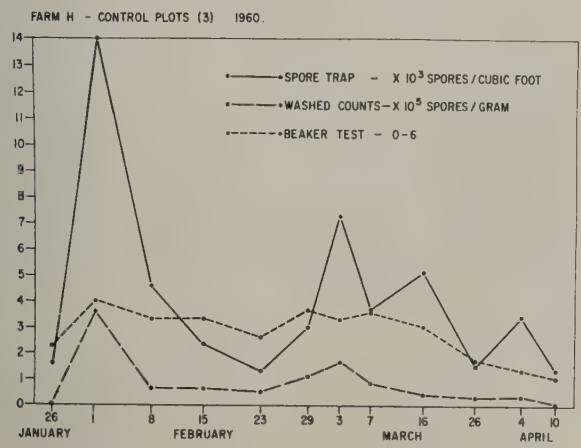
Figs. 3a (upper) and 3b (lower).—Mean of spore trap counts of *P. chartarum*, rainfall, sunshine, grass minimum temperature, and percentage relative humidity, Ruakura Sheep Farm, 1960 and 1961.

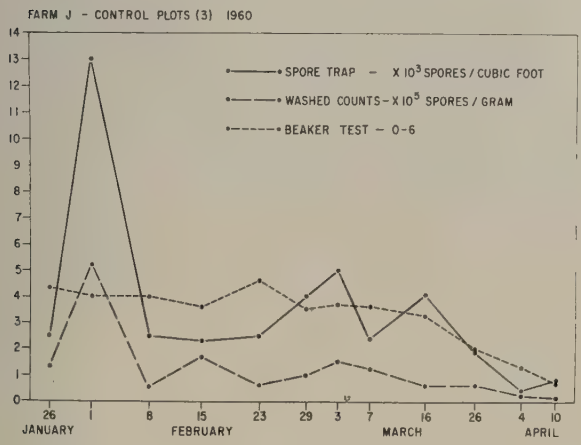
Peak spore counts on 1 March, 17 March, and 21 March occurred on days with bright sunshine, and were preceded by dull days with high atmospheric humidity. A rapid decline in spore numbers after a peak count appeared to follow (after a lag phase) a decline in temperature and a return to drier weather, e.g., at the end of February and in mid-March. The relation between weather conditions and fluctuation in spore numbers at the beginning (January to mid-February), and the end, of the period (April and May) is not clear.

On two Waikato sheep farms trends in spore numbers on herbage were followed by spore trap and haemocytometer counts (washed herbage counts) (Figs. 4a and 4b*). Spore trap and wash counts were noted to reach their maximum value on 1 February on these farms, which coincided approximately with the first noticeable rise in spore numbers on Ruakura Sheep Farm (Fig. 1). Severe facial eczema occurred in animals grazing the herbage on these plots at Ruakura up to 10 days after the spore trap peak count of 13,000 or 14,000 on 1 February.

Spore counts were not made frequently enough over this peak period on outside farms or on Ruakura Sheep Farm to follow precisely day-to-day fluctuations in spore numbers. A peak count

* Within their limited scale "beaker test" values showed some agreement with spore counts. (See Clare, N. T.; Sandos, J.; Percival, J. C. 1959: *N.Z. J. agric. Res. 2:* 1087.)


FARM H - CONTROL PLOTS (3) 1960.
SPORE TRAP - X 10³ SPORES / CUBIC FOOT
WASHED COUNTS - X 10⁵ SPORES / GRAM
BEAKER TEST - 0-6
JANUARY
FEBRUARY
MARCH
APRIL


FARM J - CONTROL PLOTS (3) 1960
SPORE TRAP - X 10³ SPORES / CUBIC FOOT
WASHED COUNTS - X 10⁵ SPORES / GRAM
BEAKER TEST - 0-6
JANUARY
FEBRUARY
MARCH
APRIL

Figs. 4a (upper) and 4b (lower).—Spore trap and haemocytometer counts and beaker test gradings on two Waikato farms, 1960.

of 13,000 did occur on 2 February on other experimental areas, at Ruakura Animal Research Station, which were regularly spore-trapped, and severe symptoms of facial eczema were noted soon after in animals grazing the herbage over the peak count period.

1961 SURVEY

Methods

Spore trap and counting techniques

As in 1960, spore-trapping was done in 1961 with a Brook mobile spore trap. This was modified by the addition of a metal

windshield to reduce the effect of cross-winds. A metal gauze was fitted over the air intake to reduce the numbers of fragments of plant material which might be deposited on the sticky slide. Trap runs in paddocks were marked by pegs on the most uniform portion of herbage available, as it had been found in 1960 that a deviation from a fixed run could result in variations in spore counts. Difficulty was experienced in counting the numbers of spores on trap slides when the count exceeded 50,000 spores per cu. ft. of air. In such cases shorter runs were made on the trapping line and the necessary adjustment incorporated when expressing the estimated count. The counting technique employed was identical to that used in 1960.

Four paddocks on each of six farms in the 1960 survey at Morrinsville, Maungatautari, Ngaruawahia, Waharoa, Te Awamutu, and Tuhikaramea were trapped in 1961. Two slides were taken on each paddock on outside farms in the survey every alternate day when weather permitted, from 16 January to 7 May. Eight paddocks (2 slides per paddock) on Ruakura Sheep Farm were trapped daily when weather permitted.

Results

From the beginning of 1961 until 20 February 1961 the spore count was low on the herbage on paddocks on Ruakura Sheep Farm (Fig. 3b). Between 20 and 23 February 1.1 in. of rain fell, coincident with dull weather, and the grass minimum temperature rose rapidly to 62.5°F on 24 February. The spore count also rose rapidly from 900 on 24 February to a peak of 13,800 spores per cu. ft. of air on 27 February during a period when no rain fell, and the relative humidity declined from 96 per cent on 24 February to 75 per cent on 27 February. This increase in spore count followed (with a lag) an increase in grass minimum temperature.

Although the grass minimum temperature remained above 56°F between 24 February and 6 March, it declined from 62°F on 25 February to 57°F on 28 February, and the decline in spore count between 27 and 28 February may be the result. After 0.02 in. of rain recorded on 1 March and a slight increase in the grass minimum temperature (56°F on 1 March to 58°F on 2 and 3 March) and falling percentage relative humidity, a slight increase in spore count was noted. Between 3 March and 6 March 0.82 in. of rain fell and on 5 March no spore counts could be made because of the wet state of the herbage. A rapid decline in grass minimum temperature from 62°F on the 6th to 40°F on the 7 March was closely followed by a rapid decline in spore count.

A small spore count peak on 18 March occurred during bright dry weather and followed a rapid decline in percentage relative humidity between 17 and 18 March. The next spore count peak on 27 March was preceded by a dull period with rain on 22–25 March, and a grass minimum of 62°F on 24 March; it occurred during bright dry weather after declining percentage relative humidity. The

final high peak spore count of the season on 19 April occurred during a period of bright weather and followed prolonged rain (14–18 April), an increase in grass minimum temperature to 65°F on 17 April, and a decrease in percentage relative humidity from 97.5 per cent on 18 April to 76 per cent on 19 April.

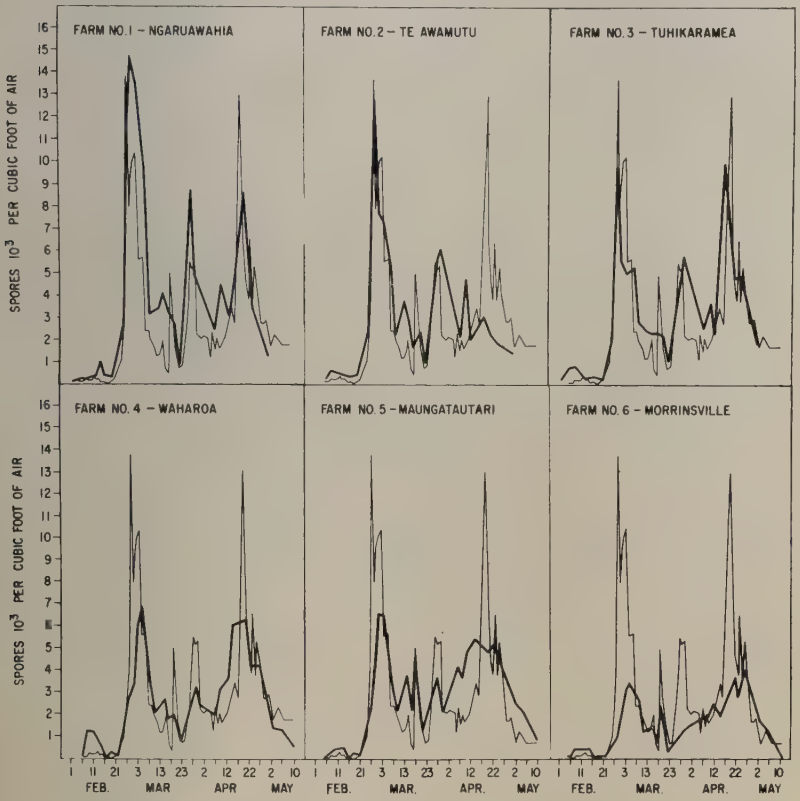

FARM NO. 1 – NGARUAWAHIA
FARM NO. 2 – TE AWAMUTU
FARM NO. 3 – TUHIKARAMEA
FARM NO. 4 – WAHAROA
FARM NO. 5 – MAUNGATAUTARI
FARM NO. 6 – MORRINSVILLE
SPORES 10^3 PER CUBIC FOOT OF AIR
SPORES 10^3 PER CUBIC FOOT OF AIR
1 11 21 3 13 23 2 12 22 2 10
FEB. MAR APR MAY

Fig. 5.—Mean of spore trap counts on Ruakura Sheep Farm (thin line), compared with means of 6 Waikato farms (dense line), 1961.

A comparison between the mean spore count of 16 slides taken on 8 paddocks on Ruakura Sheep Farm and the mean spore count of 8 slides taken on 4 paddocks on each of the 6 Waikato farms is given in Fig. 5. All outside farms and Ruakura Sheep Farm showed the first major spore-count peak in the 6-day period starting on 27 February. Spore counts on all farms showed a steady daily increase after 20 February until the peak spore count on each was reached. The magnitude of this peak (9,000 to 14,000 per cu. ft.) was similar to Ruakura Sheep Farm and on Farms 1, 2, and 3, and appreciably lower on Farms 4, 5, and 6 (3,400 to 6,900 per cu. ft.) Farms

Nos. 1, 2, and 3 and Ruakura Sheep Farm showed a second increase in spore count starting on 22 March and reaching a peak of 5,500 to 8,800 spores per cu. ft. on 28 or 29 March. Farms 4 and 5 showed a lower peak on 28 March. The final major peak count of the season was noted clearly on Farms 1, 3, 4, 5, and 6 (and less obviously on Farm 2) from 14 to 26 April. Following this final major peak, spore counts declined steadily to a low level.

On Farm No. 2 regular spore trapping was done from March onwards to compare spore counts on fixed runs on 3 different parts of one of the paddocks. These runs were as follows:—

(a) A sloping portion which tended to dry out and "burn" in summer.

(b) An intermediate area.

(c) The normal portion of the paddock.

The spore counts obtained from slides taken from these areas are shown as graphs in Fig. 6.

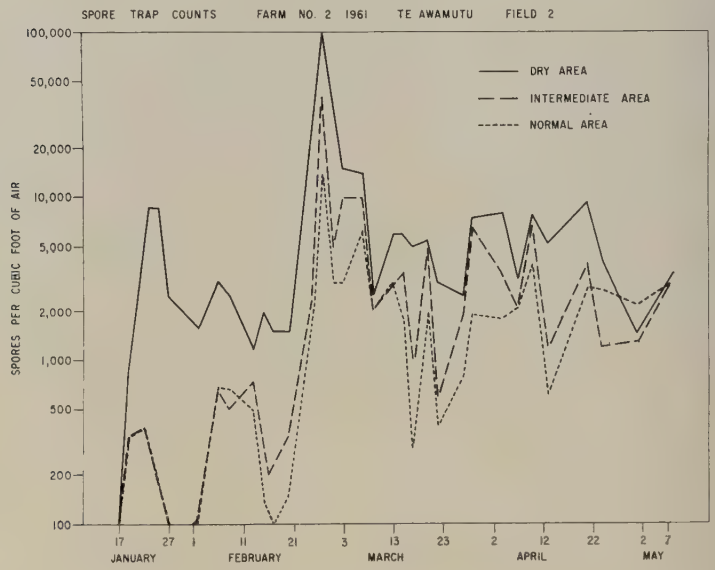
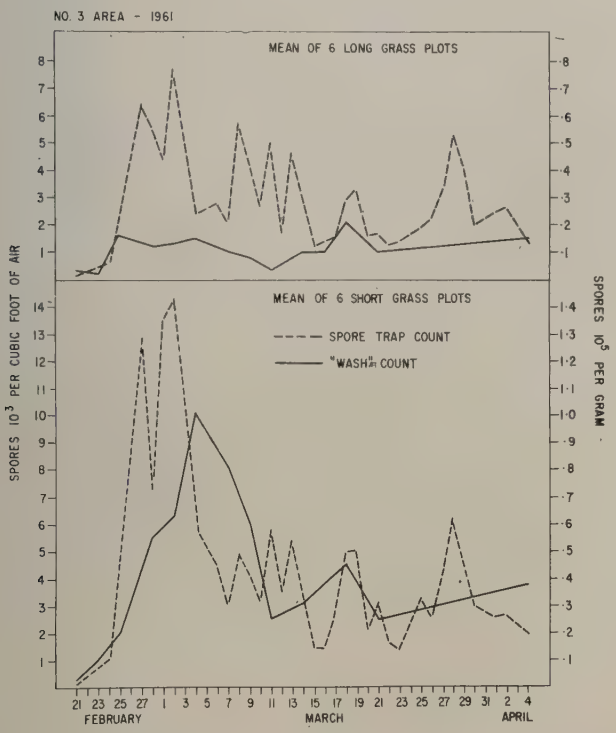


Fig. 6.—Spore trap counts, difference within a non-uniform paddock, 1961.

There was a marked tendency for spore counts to fluctuate in a similar manner on all three runs. Except at the beginning and end of the sampling period, when "burning" and litter production were at their minimum on the dry area, this latter area gave a higher count than the normal and intermediate areas.


NO. 3 AREA - 1961
MEAN OF 6 LONG GRASS PLOTS
MEAN OF 6 SHORT GRASS PLOTS
SPORE TRAP COUNT
"WASH" COUNT
SPORES 10^3 PER CUBIC FOOT OF AIR
SPORES 10^5 PER GRAM
21 23 25 27 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 2 4
FEBRUARY
MARCH
APRIL

Figs. 7a (upper) and 7b (lower).—Spore trap and wash count means on "long" and "short" grass plots, 1961.

Six long grass and 6 short grass plots (set-stocked with lambs) of a grazing management experiment at Ruakura (Smith *et al.* in press) were spore-trapped daily. Haemocytometer counts on washed herbage (wash counts) from these plots were also made throughout the season (Figs. 7a and 7b). Fluctuations in spore-trap counts followed very closely the same trends on both long and short herbage throughout the season. At the first spore-trap count peak on 27 February to 2 March the count on the short grass plots was approximately twice that on the long grass. In the field the distinction between long and short grass plots was apparent at this spore count peak period, but thereafter the distinction declined, until some of the "long" plots showed short grass areas and some of the "short" plots had patches of long grass growing in them. Although for the remainder of the season a higher spore-trap count was obtained on the short grass than on the long grass, the difference was slight compared with the difference at the peak period. A situation similar to that noted in spore-trap count is shown when the wash counts on the two types of herbage are compared.

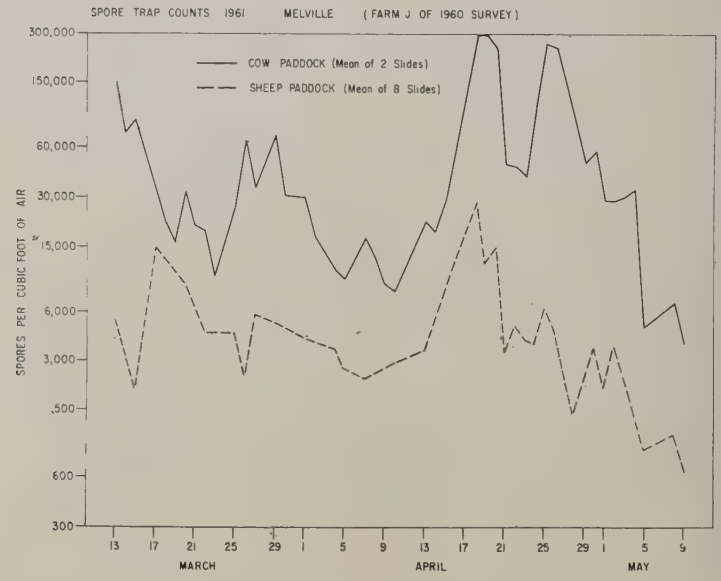


Fig. 8.—Spore trap counts on cow paddock (lightly grazed) and adjacent sheep paddock (closely grazed), 1961.

Spore-trap counts were made on sheep and cow paddocks on a farm at Melville (Farm J in the 1960 Survey) from 13 March to 10 May (Fig. 8). The sheep paddock was closely grazed. The cow paddock was under-grazed, and a large amount of detached litter accumulated at the sole, and attached litter collected round the bases of the grass plants. Throughout the sampling period counts on the cow paddock remained much higher than on the close-grazed sheep paddock, although the fluctuations in spore numbers on both followed the same trends. In April the highest count (300,000 spores/cu. ft.) in the whole survey was encountered on the cow paddock.

DISCUSSION

In the 1960 Survey the trend in the spore counts on 22 paddocks on Ruakura Sheep Farm agreed well, although peak and base counts differed widely in magnitude. The mean of the counts on the paddocks gives a good picture of the trends in spore counts during the season. Because of unfavourable weather, which hampered regular spore-trapping on the Waikato farms in the survey, insufficient spore count data available, but peak spore trap counts on two Waikato farms coincided with peak spore trap counts on those farms and on Ruakura Sheep Farm.

In the 1961 survey spore-trapping was done more frequently than in 1960, on a smaller number of paddocks. Trap runs were on fixed transects of uniform soil type and herbage, as marked

differences in spore counts were found to result from trapping different areas of the same paddock. Trends in spore counts on Ruakura Sheep Farm and on the 6 Waikato farms were similar and concurrent, although the peak and base counts between peaks varied in magnitude.

Two sources of variation in spore count were noted in the 1961 survey. Short grass plots, while they remained short, gave a higher spore count than long grass plots. As the long grass became opened up by grazing, and long grass patches developed in the short grass, the spore count difference became smaller. The fluctuation in the spore count showed the same trend in the two types of herbage. On the other hand, a paddock of short sheep pasture kept short by hard grazing was found to have a consistently lower count throughout the season than a neighbouring long grass paddock with much litter resulting from persistent under-grazing. Again trends in spore numbers were concurrent on both types of herbage.

It is possible to relate some of the salient features in the fluctuation in spore count on Ruakura Sheep Farm to meteorogical records. This can be done more readily on the 1961 survey data than on those of 1960, as the fluctuations in counts were more marked in 1961. In 1960 a peak count on 1 March in sunny weather was recorded 3 days after a 6-day period of dull wet weather during which a grass minimum temperature above 60°F was maintained for 3 days. The peak count on 17 March in sunny weather again occurred 2 days after a 5 day period of dull wet weather, during 3 days of which the grass minimum was maintained above 55°F. In 1961 there were 3 marked spore count peaks on 27 February to 3 March, 27 to 29 March, and on 1 April. All peak counts were preceded by wet weather, and occurred on sunny days during or just after a period when the grass minimum temperature exceeded 55°F. The duration and intensity of the peaks appear to be related to the duration of the higher grass minimum temperature. A feature of all 3 peak periods in 1961 was the decline in relative humidity in the 2–3 days preceding the peak count. In 1960 and 1961 a lag period of 2 to 4 days was interposed between the peak count and the previous cardinal temperature. The spore count fell away after peaks, following a rapid decline in temperature, or after rain.

The weather conditions which favour the free collection of spores by trapping are probably not those which favour sporulation. It is significant that all the 7 marked spore count peaks in 1960 occurred on dry days when there were more than 5 hours of bright sunshine (mean 9 hours). In 1961, when peak counts were more clearly defined, 5 peaks occurred on days when bright sunshine hours exceeded 3½–mean 8 hours. At all peak counts except that of 21 March 1960 (a relatively low count of under 2,000, which occurred in bright weather after a heavy thunderstorm) the weather was dry (relative humidity below 80 per cent). On 18 March 1961 spore counts increased rapidly after 4 days of very bright dry

weather (mean sunshine—11 hours) when low grass minimum temperatures and low relative humidity had been recorded. Most peak counts were preceded by a period of wet warm weather which could have favoured rapid sporulation.

It appears from the results that a marked peak count does not directly indicate rapid sporulation. It does indicate that abundant readily-detached spores are available for dispersal. These may be deposited on green leafage, which may be ingested by the grazing animal, so that peak counts indicate the likely toxicity of the herbage.

CONCLUSIONS

The trends in spore trap counts of *P. chartarum* on herbage of reference paddocks can give a reliable guide to the trends on paddocks in the surrounding farming area.

Trends in spore counts may be similar on short or long herbage or on droughted or normal herbage.

Spore count levels may differ markedly on different paddocks at a particular time.

Major spore trap count peaks are preceded by moist warm weather favourable for fungal growth; these peaks occur during dry weather probably not favouring growth but providing the desiccating conditions favourable for spore liberation and dissemination.

Although spore trap counts afford a rapid means of estimating the number of spores available for dispersal, they do not directly estimate the rate of fungal development or sporulation.

The presence of a danger period for facial eczema for grazing animals may be inferred from a sharp upward trend in spore trap counts following moist warm weather.

A method is required for the rapid direct estimation of spore load on herbage eaten by the grazing animal.

ACKNOWLEDGMENTS

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REFERENCES

Anon. 1961: Facial eczema—spore count investigation. *N.Z. J. Agric. 102:* 535–7.

Brook, P. J. 1959: A volumetric spore trap for sampling pasture. *N.Z. J. agric. Res. 2:* 690–3.

Clare, N. T. (in press): The estimation of sporidesmin. *Symp. Facial Eczema, N.Z. Microbiol. Soc.*

MITCHELL, K. J.; WALSHE, T. O.; ROBERTSON, N. G. 1959: Weather conditions associated with outbreaks of facial eczema. *N.Z. J. agric. Res. 2:* 584–604.

PERCIVAL, J. C. 1959: Photosensitivity diseases in New Zealand. XVII. The association of *Sporidesmium bakeri* with facial eczema. *Ibid. 2:* 1041–56.

SMITH, J. DREW; CRAWLEY, W. E.; LEES, F. T. (in press): The relationship between spore counts of *Pithomyces chartarum* from pasture herbage and facial eczema in sheep. *Symp. Facial Eczema, N.Z. Microbiol. Soc.*

SYNGE, R. L. M.; WHITE, E. P. 1959: Sporidesmin: a substance from *Sporidesmium bakeri* causing lesions characteristic of facial eczema. *Chem. & Ind. (Rev.) 1:* 1546.

THORNTON, R. H.; SINCLAIR, D. P. 1960: Some observations on the occurrence of *Sporidesmium bakeri* Syd. and facial eczema in the field. *N.Z. J. agric. Res. 3:* 300–13.

CHARACTERISTIC LESIONS OF FACIAL ECZEMA PRODUCED IN SHEEP BY DOSING MATERIAL CONTAINING *Pithomyces chartarum* COLLECTED FROM PASTURE

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ABSTRACT

Material containing a high proportion of spores of *Pithomyces chartarum* was collected from grassland herbage by a suction cleaner technique. When dosed to sheep this material produced morphological changes in tissues identical to naturally-occurring asymptomatic cases of facial eczema and to similar cases produced by the oral administration of sporidesmin.

INTRODUCTION

Liver lesions claimed to be characteristic of facial eczema have been induced in sheep by feeding cut toxic herbage; for example, by Filmer (1949) and Simpson *et al.* (1957).

The possible association of a fungus on herbage with facial eczema in grazing lambs was suggested by Percival and Thornton (1958) and Thornton and Percival (1959). This association was given further substance by Percival (1959), who reported the production of liver lesions in sheep dosed with macerated whole cultures of *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis (previously known as *Sporidesmium bakeri* Syd.). The isolation of a toxin from spores and mycelium of this fungus, capable of producing liver lesions, was accomplished by Synge and White (1959); they named the crystalline isolate sporidesmin. This substance has been administered extensively to sheep by Mortimer and Taylor (in press), who reported the production of symptoms and lesions closely similar, if not identical, to those found in facial eczema. The most specific lesions so far noted in sheep affected with facial eczema are those of intimal thickening of the hepatic arteries and portal veins. This thickening is strikingly eccentric in disposition, the fibro-occlusive lesion being in juxtaposition to injured bile-ducts (Done *et al.* 1960).

In the past, collection of *P. chartarum* spores from pastures in adequate amounts for animal experimentation has not been accomplished. Smith *et al.* (1961) succeeded, by the use of a filter chamber

attached to a suction cleaner, in collecting from herbage a sufficient quantity of spore-containing material for animal dosing.

The purpose of this paper is to present the results of an experiment in which material, collected from grassland herbage and containing a high proportion of conidia of *P. chartarum*, was dosed to sheep.

MATERIALS AND METHODS

Mycological

Material containing spores was collected from short pasture and lawn herbage at Ruakura Animal Research Station during the period 22 to 28 March 1961 using the filter chamber method (Smith *et al. loc. cit.*). The separate collections (in all amounting to 120 g of material) were combined and thoroughly mixed, and the numbers of spores of the principal species of fungi present were estimated by haemocytometer counts. These are listed below.

Fungus	Number of spores per g of debris
P. chartarum	120×10^6
Epicoccum sp.	7.5×10^6
Helminthosporium spp.	2×10^6

Smaller numbers of conidia of *Cladosporium* and *Alternaria* spp. were present. The non-fungal material consisted principally of herbage fragments and siliceous material. On the basis of estimates made with spores from ryecorn cultures* (1 g = $1{,}000 \times 10^6$ spores) *P. chartarum* spores constituted about one-eighth of the sample by weight.

Assay of toxin

When samples of spores were separated from the mixed material by the dry paper technique (Smith *et al. loc. cit.*) and assayed for sporidesmin by the azide-iodine method (Russell 1960; N. T. Clare pers. comm.) their sporidesmin content was found to be 30 μg per 100×10^6 spores.** Eluates from paper chromatograms of extracts of these spores were instilled into rabbits' eyes. The lesions which developed were identical to those produced by sporidesmin as described by Done *et al.* (1961).

* One of us (W.E.C.) found that 4 separate batches of spores of the C — isolate grown on ryecorn contained $1{,}050 \times 10^6$, $1{,}070 \times 10^6$, $1{,}090 \times 10^6$, and $1{,}050 \times 10^6$ spores per g respectively on a dry-matter basis.

Packard (1960) found that *P. chartarum* spores in water had a density of 0.79 g/ml and "air dry" 1.32 g/ml.

** The authors are indebted to Mr N. T. Clare for the chemical assay of sporidesmin in this material by the azide-iodine method. Spores of the common fungal species found with *P. chartarum*, viz. *Epicoccum*, *Helminthosporium*, *Cladosporium*, *Alternaria*, *Curvularia*, and *Penicillium* spp., were grown on ryecorn. Examination of these associated fungi, as well as of the diluting material in field collections, showed little azide-iodine activity (N.T. Clare pers. comm.).

Administration of spore material to sheep

Four Romney sheep, each aged 8 months, were maintained indoors during the facial eczema season and fed to appetite on dried grass collected in the winter and early spring. To each of 3 of these sheep 13.3 g of spore-containing material was administered by stomach tube on 3 successive days. Each daily dose was freshly suspended in 250 ml of water to which 10 ml of 5 per cent aqueous Tween 80 had been added to facilitate wetting and suspension of the spores. The remaining control sheep was similarly dosed with water and wetting agent alone.

It was estimated that each of the 3 test sheep received a total of 4,800 $\times$ 10^6 spores of *P. chartarum* containing 1.44 mg of sporidesmin as determined by chemical assay. This represented a dosage rate of 0.06 mg sporidesmin per kg body weight.

One of the test sheep died as a result of intercurrent illness before the completion of the experiment. The remaining 3 sheep were slaughtered on the 26th day from the start of the experiment and subjected to post-mortem examination.

Histological methods

Tissues were taken from the liver, kidneys, bladder, ureters, and adrenal glands and immediately placed into 10 per cent formalin,

Fig. 1.—Portal areas of the liver grossly enlarged by granulation tissue, proliferated bile-ducts, and inflammatory cells. Vacuolation of the parenchymal cells is marked in this section. The small portal veins show occlusive lesions of intimal fibrosis. (Heidenhain's Iron Haematoxylin $\times$ 100.)

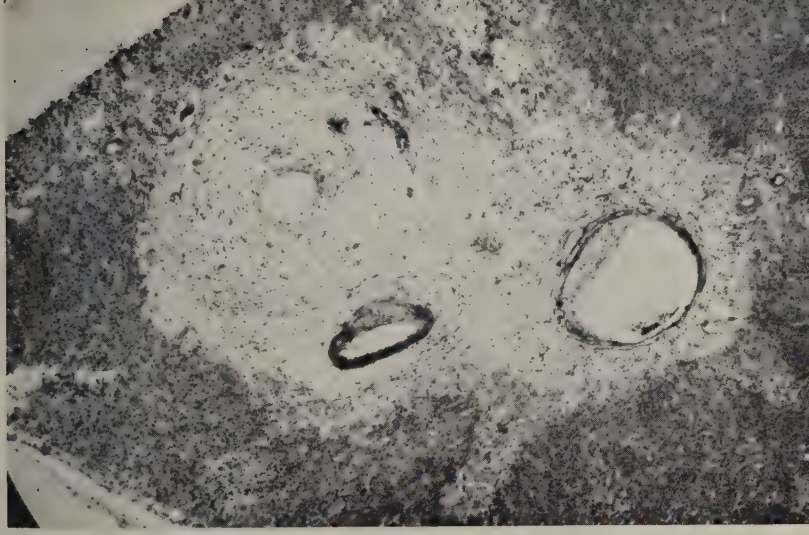

Fig. 2.—A larger portal tract in which the bile-duct (top left) is nearly occluded by granulation tissue, organising in a concentric manner. Note the relation of this bile-duct to the eccentric intimal thickening in the lumen of the hepatic artery (below) and portal vein (to the right). (Heidenham's Iron Haematoxylin × 40.)

the fixative being twice changed on subsequent days. Paraffin sections were stained with Ehrlich's Haematoxylin and Eosin, Heidenhain's Iron Haematoxylin, and a modified Weigert's method for elastica.

RESULTS

Clinical signs in dosed sheep

One animal died on the sixth day of the experiment without obvious premonitory signs. Neither of the 2 remaining animals displayed icterus, nor were they photosensitive when placed out-of-doors in direct sunlight.

Post-mortem examinations

The sheep which died of intercurrent illness in the first week of the experiment exhibited no macroscopic liver lesions. Upon opening the thorax of this cadaver, a chronic pleuritis and pneumonitis was revealed, and the marked adhesions of the left lobes of the lung to the thoracic wall were firm, organised, and of long duration. When the lungs were incised, an acute fulminating pneumonia, probably stemming from the chronic involvement of the respiratory system, was found to be the likely cause of the animal's premature death. Evidence of recent inhalation pneumonia was not found.

The 2 remaining dosed sheep, slaughtered on the 26th day of the experiment, showed macroscopic liver lesions which were more advanced in one sheep than in the other.

The liver showing the milder lesions was slightly more firm than is normal and the lobular morphology was somewhat exag-

gerated, especially in the left lobe. The edges of this lobe seemed particularly fibrous. On slicing this liver, the large and medium-sized bile-ducts were found to be moderately thickened with fibrous tissue, those of the left lobe being more markedly affected. The extrahepatic bile-ducts and gall-bladder appeared normal, as did all other organs of this sheep.

In comparison, the liver of the more severely affected animal was decidedly indurated, with exaggerated lobulation throughout. This liver cut with resistance, revealing marked fibrosis of the larger intrahepatic bile-ducts to a degree which caused reduction in the size of their lumina. The medium-sized and smaller ducts, though to a less marked degree, were similarly affected in both left and right lobes. On closer examination a fine, granular fibrosis was found throughout the substance of the liver. When cut, the extrahepatic bile-ducts also showed a moderate degree of thickening, but the gall-bladder appeared normal.

The urinary bladder displayed sub-mucosal petechial haemorrhages. Cortical enlargement of the adrenal glands was noted. All other organs appeared normal.

No lesions were found in the control sheep.

Fig. 3.—A large portal vein showing a plaque of intimal thickening in one quadrant of the vessel (bottom right). This can be compared with the normal quadrant at the top of the section. The dark masses in the lumen are post-mortem blood clots. (Modified Weigert's Elastica. × 40.)

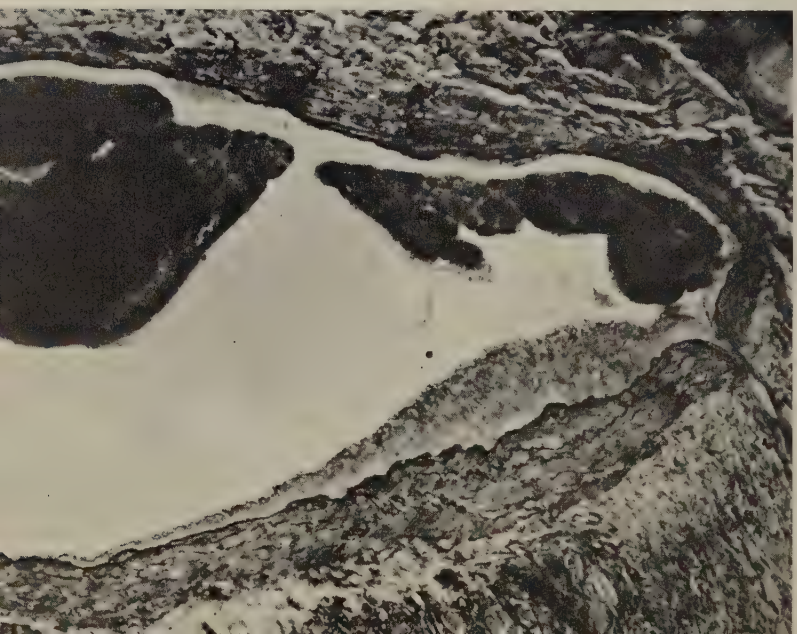

Fig. 4.—A very advanced lesion occupying a considerable part of the lumen of a portal vein in which the intimal thickening is strikingly one-sided and related to a large bile-duct below and off the field of view. The abnormal plaque shows the most dense and oldest tissue bordering the lumen. The paler area, closest to the internal elastic membrane, is recently-formed granulation tissue. The plaque is lined with endothelium, and thrombosis is not a feature. (Modified Weigert's Elastica. × 40.)

Histopathological examination

The liver of the dosed animal which died early in the experiment showed vacuolation of hepatic cells in the periportal zone of the lobules. Around the medium and larger bile-ducts were signs of early sub-epithelial oedema and lymphocytic infiltration. In some areas degeneration of bile-duct epithelium was noted, but this may have been a post-mortem change.

Lesions in the livers of the other dosed animals were well advanced, though still active. In both animals vacuolation of periportal hepatic cells was widespread (Fig. 1). In the same zone isolated hepatic cells were degenerating and were in the process of removal by hisitiocytes and polymorphs. Occasional focal necroses of the Charcot-Gombault type were to be found. Older lesions of this type showed fibroblastic activity.

The portal tracts were much enlarged, with recently-formed granulation tissue impinging on bordering lobules (Fig. 1). The lumen of the bile-ducts was largely occupied by granulation tissue undergoing organisation in a concentric fashion (Fig. 2). The lining epithelium was absent from many of the medium- and larger-sized ducts. Both portal veins and hepatic arteries showed eccentric intimal thickening, the adventitious tissue occupying that part of the vessels' lumen in closest proximity to the partly-occluded bile-

duct (Figs. 2, 3, and 4). Some degree of proliferation of the smallest bile-ducts was to be seen at the perimeters of the enlarged portal areas (Fig. 1). Inflammatory cells were still present in these areas, particularly lymphocytes, histiocytes, and, occasionally, eosinophils.

The walls of the extrahepatic bile-ducts and gall-bladder showed a similar cellular reaction, and recent sub-epithelial granulation tissue was becoming organised.

The urinary bladder of one animal displayed small sub-mucosal haemorrhages, and some granulation tissue was to be found in the lamina propria. In the same animal there were degenerative changes in the zona reticularis and hyperplastic changes in the zonae fasciculata and glomerulosa of the adrenal cortex. A heavy infiltration of eosinophils was noted in the medulla.

The liver and other organs of the control animal were found to be normal.

DISCUSSION

That the method of collection of spores was found to be practicable at a time when spore numbers of *P. chartarum* on herbage were declining, limited the amount of material collected. It was therefore necessary that the material should not be further depleted by attempting the separation of *P. chartarum* spores from the remainder, since this would inevitably result in losses of the required material. It could be objected that the mixed material which was dosed, contained, as well as conidia of *P. chartarum*, other fungal structures (mainly spores) and soil and plant particles. This extraneous material must, in any case, be ingested during grazing. The spores of those species which constituted the remainder of the fungal material have shown little azide-iodine reacting substance when compared with that present in *P. chartarum* spores.

By dosing the material as collected it was considered that sufficient sporidesmin could be present in the *P. chartarum* spores to produce morphological liver changes, if not symptomatic disease. This proved to be the case, for though liver lesions were produced, they were symptomless.

The sub-mucosal haemorrhages of the urinary bladder, the lesions of the adrenal cortex as described, and the unique intimal thickening found in the blood vessels of the liver have all been previously reported as characteristic of field outbreaks of facial eczema (Done *et al.* 1960). Similar histological lesions have also been produced in sheep orally dosed with sporidesmin. These will be described in a subsequent paper by Mortimer.

Thus the dosing of material, mainly spores, collected from grassland herbage produced morphological changes which were identical to those found in naturally-occurring, asymptomatic cases of facial eczema. This finding gives more support to the concept that it is the spores of *P. chartarum* which are the main source of sporidesmin ingested by sheep.

ACKNOWLEDGMENTS

The authors wish to thank Mr B. J. Young for the histological preparations.

REFERENCES

Done, J.; Mortimer, P. H.; Taylor, A. 1960: *Res. vet. Sci. 1:* 76.

Done, J.; Mortimer, P. H.; Taylor, A.; Russell, D. W. 1961: *J. gen. Microbiol. 26:* 207.

Filmer, J. F. 1949: *Proc. Specialist Conf. Agric.:* C320.

Mortimer, P. H.; Taylor, A. (in press): *Res. vet. Sci.*

Packard, R. Q. 1960: *N.Z. J. Sci. 3:* 412.

Percival, J. C. 1959: *N.Z. J. agric. Res. 2:* 1041.

Percival, J. C.; Thornton, R. H. 1958: *Nature, Lond. 182:* 1095.

Russell, G. R. 1960: *Ibid. 186:* 788.

Simpson, J. E. V.; Sinclair, D. P.; Swan, J. B.; Filmer, J. F. 1957: *N.Z. J. Sci. Tech. A38:* 947.

Smith, J. Drew.; Crawley, W. E.; Lees, F. T. 1961: *N.Z. J. agric. Res. 4:* 725.

Synge, R. L. M.; White, E. P. 1959: *Chem. & Ind. (Rev.) 49:* 1546.

Thornton, R. H.; Percival, J. C. 1959: *Nature, Lond. 183:* 63.

POLLINATION AND SEED-SETTING OF RED CLOVER IN SCRIM-COVERED CAGES

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ABSTRACT

A great diversity of views is held on the efficiency of the honey-bee and certain species of bumble-bee as pollinators of red clover seed crops. An attempt was made to test the efficiency in New Zealand of the honey-bee and the short-tongued *Bombus terrestris*, compared with the accepted efficiency of the long-tongued *B. ruderatus*, as pollinators of red clover. Plants were grown in scrim-covered cages, bees introduced during the flowering period, and the resultant seed crop harvested and the results noted. The experiments were carried out each year from 1954 to 1958 inclusive. The results showed that great variation occurred from year to year and factors other than pollinating insects were involved. The differences between the species over the years were not consistent. *B. terrestris* and the honey-bee are both shown to be capable of effecting an appreciable degree of red clover pollination. Evidence pointed to *B. terrestris* as the principal agent in the pollinating of red clover seed crops in the Lincoln College area in 1958. The results indicate that the practice of placing honey-bee colonies in the vicinity of red clover seed crops is sound.

INTRODUCTION

The main object of this investigation was to endeavour to assess the relative efficiency of the honey-bee (*Apis mellifera*) and two introduced species of bumble-bee (*Bombus ruderatus* and *Bombus terrestris*) as pollinators of red clover. To this end results were collected for each of the five years 1954 to 1958 from experiments carried out inside scrim-covered cages on the Lincoln College farm at Lincoln and checked in 1955 and 1958 against results of seed setting on red clover crops growing in the near vicinity.

Red clover flowers are pollinated by insects when collecting either nectar or pollen provided sufficient pressure is applied to the petals to cause tripping. Almost without exception this is performed only by bees, both bumble-bees and honey-bees. In red clover there is no naturally occurring side opening whereby bees can obtain nectar without tripping the flowers as may occur sometimes when honey-bees are collecting nectar from lucerne flowers. There has been much controversy concerning the relative efficiency of the different species of bumble-bee and the ability of the honey-bee to pollinate red clover. Butler *et al.* (1956) refer to the effectiveness of bumble-bees in general

as pollinators of red clover, suggested by the increase in seed production in New Zealand after their introduction to that country; but these investigators state that their value is greatly reduced by the fact that certain species with short tongues (e.g., *Bombus lucorum, B. terrestris*) bite holes near the bases of the corolla tubes and thus obtain nectar without effecting pollination. In the U.S.S.R. it has been estimated that 15 per cent of all bumble-bee visits to red clover are of this "robbing" kind. Not only does this fail to pollinate the flowers but it enables or directly encourages honey-bees to obtain nectar in the same manner, and in fact certain European investigators found that the number of "robber" honey-bees was correlated with the number of the robber bumble-bees. Williams (1925), studying red clover pollination in Wales, observed nine species of bumble-bees and the honey-bee visiting the crops and remarked that, contrary to expectations on the basis of observations made by various investigators on the Continent and in the United States, that the contribution made by honey-bees towards the fertilisation and seed production of red clover at Aberystwyth and in the seed-growing centre in Montgomeryshire was practically negligible. Bird (1944), studying seed setting in red clover in eastern Canada, found that although density of stand and profusion of bloom may determine the lower and upper limits of seed yield per acre, wide fluctuations between such limits from season to season and from place to place may result from variation in the set of seed as indicated by the average number of seeds per head in samples drawn from the ripened crop. The number of seeds per head was dependent in turn, he found, upon the number of florets per head, their condition at the time of pollination and fertilisation, and the number and activity of pollinating insects. He concluded that the number of bumble-bee visitors was a much more important factor in seed setting than number of honey-bee visitors or number of florets per head. Cumber (1953) reached much the same conclusion about honey-bees and contended that, although it is recognised that under some conditions the honey-bee may effect a measure of pollination, the bumble-bee is a much more efficient pollinator and is the main agent wherever suitable species are abundant. Certain factors, he stated, may increase the efficiency of the honey-bee as a pollinator but few would advocate its use except as a last resort, for results to date are not encouraging.

Opposing views are held about the purpose of honey-bee visits to red clover crops. Dunham (1939), investigating honey-bee activity in Ohio, U.S.A., designed his experiments in the conviction that honey-bees devote nearly all their time to gathering pollen from red clover blooms and that practically no nectar is gathered. Butler *et al.* (1956), on the other hand, state that "We now know that large numbers of honey-bees do sometimes visit red clover flowers and produce considerable quantities of honey in consequence." Bohart (1957) explains the discrepancies in observations on honey-bee activity as probably resulting from variable complexes of competing bloom, as well as variations in the nectar content of red clover.

In New Zealand the value of the honey-bee as a pollinator of red clover is still hotly disputed. This is not the case with the long-

tongued bumble-bee *B. rudcratus* which, when sufficiently numerous, is regarded as an excellent pollinator of red clover crops. The short-tongued *B. terrestris*, which sometimes damages the base of the corolla in its search for nectar, is often condemned as an unfortunate importation. Recent surveys (Dumbleton 1948; Gurr 1957) show that these are the only bumble-bees widely distributed throughout the red clover seed-producing areas of New Zealand. The only other species present in the country are *B. subterrancous* subsp. *latreillellus* and *B. hortorum* —both of which are extremely scarce, unevenly distributed, and largely confined to the foothill regions of the southern part of the South Island.

METHOD

Ten contiguous cages, each 6 ft sq. and 6 ft 6 in. in height, provided with a narrow door, and constructed of a wooden framework on a concrete foundation, were erected in a single row and numbered consecutively. The cages were covered with clear nylon gauze of 14 mesh. Clonal red clover was planted out so that all the parent plants were represented in each cage. In addition to red clover, other bee forage plants, including antirrhinum scarlet runner bean, white clover, and lucerne were planted in each cage to provide an alternative source of pollen or nectar as desired. During the summer months the clover sometimes required cutting in the early part of the season and watering in the later part.

The pollination experiments were carried out during the months of February and March and the seed heads left to ripen until May before being harvested. A strong but small colony of honey-bees, provided with an adequate food store and access to water, was introduced in a hive and left throughout the two months' period in one of the cages and given access to an adjoining cage by removing a portion of the scrimmed partition. The colony was still healthy and strong when removed at the end of the two months' pollination period each year. Bumble-bees were collected regularly and placed in their respective cages every few days during the pollination period. Sometimes bumble-bees lived for a fortnight or longer, but at other times mortality was high and introductions had to be made at fairly frequent intervals in order to keep up a population of not less than four individuals per cage. *B. terrestris* was plentiful each year and throughout the whole pollinating season. This species could be collected most readily from *Cosmos*, *Escallonia*, flowering crops of broccoli, and Scotch thistle. *B. ruderatus* was a scarce species in each of the five years under review. In 1954 and 1958 it did not disappear until early April, but in the three intervening years it had practically disappeared by mid-March. It could be collected most readily from red clover flowering along roadsides and hedges and from scarlet runner beans. It is a much more agile bee than *B. terrestris* and more difficult to catch. From observations made while collecting bumble-bees it was estimated that the relative proportions of the two species in the neighbourhood of Lincoln College were in the vicinity of 20 or 30 *B. terrestris* to one *B. ruderatus*.

B. terrestris bumble-bees, the one-striped, short-tongued species, occupied cages 1, 5, and 7. The honey-bees occupied cages 2 and 3,

while *B. ruderatus* bumble-bees, the two-striped, long-tongued species, occupied cages 4, 6, and 8. In 1954 one of the remaining cages was left closed without the introduction of any pollinating insects. Also in that year the other remaining cage was reserved for regular introductions of the white butterfly (*Pieris rapae*). In 1955 one of the cages was left permanently open for insects of all sorts to enter and leave at will. In all cases the various bee species occupying the different cages seemed to settle down fairly quickly and behave in the normal manner of an insect visiting flowers. The red clover plants, however, behaved differently from plants in the open. Although vegetative growth was usually very pronounced the amount of blooms formed varied greatly from season to season.

In order to compare results obtained from scrimmed cages with those from crops in the field, opportunity was taken in 1955 and again in 1958 to collect seed heads from red clover crops growing on land of Crop Research Division, D.S.I.R., at Lincoln. Those crops were approximately one mile distant from the experimental cages and about the same distance away from any apiary in the district.

RESULTS

A summary of the results obtained from the cages for the years 1954 to 1958 inclusive and from the open-pollinated crops growing on the Crop Research Division land at Lincoln for the years 1955 and 1958 is set out in Table 1.

TABLE 1. AVERAGE NUMBER OF SEEDS PER HEAD PRODUCED BY RED CLOVER PLANTS GROWING IN SCRIM-COVERED CAGES WITH POLLINATING AGENTS CONTROLLED, AND ON OPEN POLLINATED CROPS

(Averages based on the order of 100 heads)

Pollinating agent	1954	1955	1956	1957	1958
B. terrestris	9.8	34.3	22.9	9.8	67.0
B. ruderatus	11.6	40.3	49.5	10.5	48.4
Honey-bee	4.7	35.6	24.0	6.0	45.4
Open pollinated crops	—	91.8	—	—	66.3

A statistical analysis of the results shows mainly that:

(a) large variation occurred from year to year; and

(b) no differences between the species were consistent over the years.

The seed set in the closed cage in 1954, where no insects had access to the plants, was nil. That for the cage where white butterfly was introduced regularly throughout the flowering season was also practically nil. The cage which was left open throughout the flowering season in 1955 had a moderately good set of seed but not quite as good as in any of the cages containing bees.

DISCUSSION

Since sufficient pollinating insects were introduced to the cages each year the large differences between the years were due apparently in this case to factors other than pollinators. In 1954 and 1957 the number of seeds set was very poor for all kinds of bees compared with those for the years 1955, 1956, and 1958. Since final yield of seed depends in large measure on the number of seeds set per head, then 1954 and 1957 were years of low yield compared with 1955, 1956, and 1958. Variations of this order may not be uncommon in the field. Hawkins (1956) states that the yield of red clover seed grown on the same farm on the Essex-Suffolk border in England was recorded for 26 years between 1914 and 1948 and varied from 67.2 lb to 716.8 lb per acre.

The results show clearly that there is no doubt about the ability of the honey-bee to pollinate red clover. Although in each of the years under review it proved less valuable than *B. ruderatus*, this was to be expected because red clover can be regarded as very high up the list of preferred foods of that bumble-bee, whereas it is very lown down on the list of preferred foods of the honey-bee. Nevertheless, the honey-bee with an ample reserve of food in the hive visited the clover blooms and effected pollination. *B. terrestris* is also shown to be an effective pollinating agent of red clover, although that plant is by no means near the head of the list of preferred foods for that species of bee.

The figures for the year 1958 are particularly illuminating. *B. ruderatus* was extremely scarce throughout the whole season and much searching had to be done to obtain sufficient individuals to place in the cages. In fact the species had practically disappeared altogether by mid-March and no further introductions were made. That doubtless accounts to some extent for the low figure of 48.4 for that species compared with 67.0 for *B. terrestris*. The year 1958 was the only year that it failed to surpass the honey-bee by an appreciable amount.

The open pollinated crops in 1958 gave an average figure of 66.3, almost identical to the *B. terrestris* figure of 67.0. Those crops were grown on an area of land one mile distant from any apiary in a district where *B. ruderatus* was extremely scarce throughout the season and practically non-existent from mid-March onwards. *B. terrestris* was abundant throughout the season; hence the inference is that *B. terrestris* was the principal, and most probably almost the sole, pollinating agent.

CONCLUSIONS

B. ruderatus is an efficient pollinator of red clover and is especially suited to the performance of this function because of its adequate tongue length and its distinct preference for red clover flowers. *B. terrestris* and the honey-bee can readily obtain pollen from red clover but both may experience difficulty in reaching the nectar. Neither of those two types of bee has a very high preference for red clover flowers as a food plant. Nevertheless, as this investigation has shown, both *B. terrestris* and the honey-bee can and do pollinate red

clover to quite an appreciable extent. The placing of colonies of honey-bees in close proximity to red clover crops to encourage their visiting the flowers and so effecting pollination must be regarded as sound practice. Any suggestion that *B. terrestris* was an unfortunate introduction to New Zealand cannot be supported. There is some evidence which points to this species having been the chief pollinator of red clover crops in the Lincoln College area in 1958.

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REFERENCES

Bird, J. N. 1944: Seed setting in red clover. *J. Amer. Soc. Agron. 36:* 346–57.

Bohart, G. E. 1957: Pollination of alfalfa and red clover. *Annu. Rev. Ent. 2:* 355–80.

Butler, C. G.; Free, J. B.; Simpson, J. 1956: Some problems of red clover pollination. *Ann. appl. Biol. 44:* 664–9.

Cumber, R. A. 1953: Some aspects of the biology and ecology of humble-bees bearing upon the yields of red clover seed in New Zealand. *N.Z. J. Sci. Tech. B34:* 227–40.

Dumbleton, L. J. 1948: Bumble-bee species in New Zealand. *Ibid. A29:* 308–12.

Dunham, W. E. 1939: Collecting red clover pollen by honey-bees. *J. econ. Ent. 32:* 668–70.

Gurr, L. 1957: Bumble-bee species present in the South Island of New Zealand. *N.Z. J. Sci. Tech. A38:* 997–1001.

Hawkins, R. P. 1956: A preliminary survey of red clover seed production. *Ann. appl. Biol. 44:* 657–64.

Williams, R. D. 1925: Studies concerning the pollination and fertilization and breeding of red clover. *Bull. Welsh Pl. Breed. Sta. H4.*

FACTORS INFLUENCING THE GROWTH OF *Pithomyces chartarum* IN PASTURE

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ABSTRACT

An examination of factors which could influence the growth of *Pithomyces chartarum* and the development of facial eczema toxicity in pasture indicated that:

1. Growth and sporulation on dead grass leaves and stems is most rapid under warm (70°F to 80°F) moist conditions. It is retarded by prolonged exposure to high levels of moisture around the dead herbage, and considerably accelerated on tissues with a high content of soluble organic material, e.g., freshly dead, well-nourished leaves.

2. The ability of spores to adhere to live leaves is directly related to the wettability of the leaf surfaces.

3. The wettability of the lower epidermis of ryegrass leaves is high. The upper epidermis of ryegrass leaves and both surfaces of the leaves of paspalum and cocksfoot are difficult to wet. The lower epidermis of white clover leaves is moderately wettable.

4. Spore germination is greatly stimulated by cell exudates from damaged areas on live leaves of ryegrass or paspalum provided the leaves are moist. Dilute juice pressed out from ryegrass leaves has a similar effect on spore germination.

5. As judged by nitrogen analyses, considerable quantities of soluble organic material are readily available at the surface of immature leaves of ryegrass if they are wetted after being grown under warm moist conditions. This is not the case with mature leaves.

6. Germination of spores followed immediately by a period of rapid mycelial growth in dilute ryegrass juice can lead to a significant rise in facial eczema toxicity within 15 hours.

INTRODUCTION

As the development of facial eczema toxicity in a pasture is closely correlated with the presence of the fungus *Pithomyces chartarum* (Berk & Curt.), M.B. Ellis, syn. *Sporidesmium bakeri* Syd. (Percival and Thornton 1958), the investigations reported here were undertaken to examine aspects of the pasture and its environment which might have a significant influence on the development of toxicity. Special attention was paid to the influence of climatic conditions and of the leaf surface on spore germination and mycelial growth.

EXPERIMENTAL METHODS AND RESULTS

Environmental conditions influencing the development of P. chartarum *in a ryegrass pasture*

Environmental conditions favourable for the growth and sporulation of *Pithomyces chartarum* on a ryegrass sward were assessed by comparing the growth of the fungus at different temperatures and moisture levels. In experimental glass-houses boxes of ryegrass turf were held at the various combinations of temperature and moisture set out in Table 1. There were two boxes for each treatment. Prior to the start of treatments the quantity of dead and dying leaf material naturally present in this turf taken from a spring pasture was supplemented by scattering additional pieces of ryegrass tissue on to the turf. This additional "litter" consisted of pieces of freshly cut green leaves, together with pieces of flower stems and leaves taken from a sample of well-preserved hay.

A suspension of *P. chartarum* spores was sprayed on to each box of turf and a visial assessment of mycelial development was made 46 hours later. The degree of sporulation on the dead material was estimated at the end of the fourth and the sixth days. Two observers independently assessed sporulation on a 0 to 5 scale in terms of the relative area of dead tissue covered by visible black patches; five was the score for the worst affected box. In each treatment microscopic examination showed these patches to consist only of *P. chartarum* spores.

In general, visible mycelial growth was most rapid at the higher temperature and the intermediate moisture level, and greatest spore development also occurred under these conditions (Table 1). At the higher temperature, development to sporulation was limited both by an absence of free water on the leaf surfaces and by too much water on the leaves. At the end of the sixth day the adverse effect on sporulation of excess water being continuously present on the leaves was strongly apparent at both temperatures.

It was also noticed that the earliest production of spores on dead tissue generally occurred on the leaf blades and not on the flower stems, but that by the sixth day the position was reversed and most of the spore masses visible were on pieces of stem, particularly in treatments which were at the lower temperature or held continuously wet. The most rapid spore development occurred

TABLE 1. AVERAGE RATING OF DENSITY OF SPORE DEVELOPMENT IN PERENNIAL RYEGRASS TURF 4 DAYS AFTER INOCULATION WITH *P. chartarum* SPORES

Rating: 0 to 5 scale.

Moisture treatment		Temperature conditions	
		Day: 65–70°F Night: 52–56°F	Day: 75–85°F Night: 68–73°F
High moisture	Continuously	1.25 L*	1.50 S & L
Leaves thoroughly wet and dripping	Day temperatures raised approx. 5°F on 3rd and 4th day	1.25 L*	3.25 L & S
	Night temperatures lowered to 40–50°F on 3rd and 4th night	0.75 L*	2.25 L
Moderate moisture	Continuously	0.50 L*	4.00 L & S
Leaves wet most of the time but not dripping	Day temperatures raised approx. 5°F on 3rd and 4th day	1.50 L*	4.50 L & S
	Night temperatures lowered to 40–50°F on 3rd and 4th night	0.25 L*	2.75 L
Humid conditions	Continuously	0.50 L	3.25 L & S
After spore germination period, leaves in a high-humidity atmosphere but free water only occasionally present on leaf surface.	Day temperatures raised approx. 5°F on 3rd and 4th day	0.75 L	3.00 L & S
	Night temperatures lowered to 40–50°F on 3rd and 4th night	0.0	2.50 L & S

L. Sporulation almost entirely on leaves.
L & S. Sporulation most vigorous on leaves, but considerable amount on stems also.
S & L. Sporulation most vigorous on stems, but considerable amount on leaves also.

*In general, sporulation was largely confined to older dead leaves, but in each treatment marked with an asterisk * spore development was mainly on freshly cut green leaves which had been put among the herbage just before inoculation.

on the freshly cut pieces of green leaf at the lower temperature, but at the higher temperature fastest development occurred on leaves which had been made into hay or otherwise dried and aged.

It was concluded from these observations that litter with a reasonably high content of soluble organic material stimulates rapid sporulation, but that, as such litter can decay rapidly and become soggy, the spore masses generally disappear within a few

days. Where litter contains less soluble organic material but a higher proportion of cellulose and lignin, which makes it more durable, sporulation is slower but the spore masses may remain present for a considerable time.

Other points noted from work carried out in other glasshouses were that where extensive development of the fungus had occurred in dead litter prior to its being held under dry conditions for a considerable time, the first major flush of sporulation took place about 30 hours after a subsequent onset of warm moist conditions. When such infected litter was maintained in conditions which were rather too wet for appreciable development of the fungus and was then allowed to dry out under warm conditions, such as occurred when the sun shone into the base of the sward, an efflorescence of spores appeared in the hour or two when the dead leaves were being warmed up by the sun and before the reduction in moisture had become sufficient to dry them and check fungal development.

The retention of spores on live leaves

Live leaves normally constitute the major portion of a sheep's intake of grass from a pasture. Except where there is a close admixture of live and dead leaves, an animal eating relatively fastidiously may not ingest appreciable quantities of dead material. In this case any fungal spores and mycelium present on live leaves might well be important sources of toxicity if they occur in large enough quantities. Spores could be transferred from the litter at the base of a pasture onto the live leaves above by air movement, by being splashed up in water droplets, or by disturbance resulting from animal treading and grazing.

In the present investigation the first situation was simulated by puffing dry spores at leaves and the second by spraying a suspension of spores in water onto them. Disturbance by animals could cause movement of both dry spores and those suspended in water droplets. Factors influencing the retention of these spores by leaves were investigated in three ways.

I. *Adhesion of dry spores to lower epidermis.* The adhesion of dry spores to the lower epidermis of the leaf was studied in a range of grasses. Unless otherwise stated, all plants were grown in a glasshouse at a relative humidity of approximately 70 per cent prior to treatment. Spores were then puffed on to individual leaves, and an estimate was made of the numbers retained as a result of the following treatments:—

A. Leaves left dry for 24 hours prior to application of spores.

B. As "A", but immediately after application of spores the leaves were sprayed with water for 30 seconds.

C. Plants placed in a water-saturated atmosphere at 80°F in a growth chamber for 2½ hours before spore application.

D. Leaves sprayed with water for 30 seconds, then disturbed manually to remove water not firmly adhering to them and to standardise treatment before application of spores.

E. As "D", but leaves were allowed to dry for 2 to 4 hours after the application of spores and then wetted again in an attempt to wash them off.

F. Leaves dipped in water to which detergent had been added prior to application of spores.

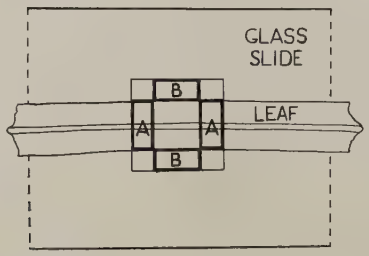

Fig. 1.—Diagram to illustrate the method used for the determination of the percentage of spores adhering to leaves. (An explanation is given in the text.)

The percentage of spores adhering to each leaf after treatment was estimated by using the technique shown in Fig. 1. Standard glass microscope slides were smeared with a thin film of petroleum jelly and the leaf clipped to the slide. Spores were then puffed onto the leaf and slide, and a microscopical examination was carried out to compare the number adhering to the leaf in region A with the number held by the petroleum jelly in region B. In the count on each individual leaf there were always well over 100 spores in the B—control—regions and frequently well over 1,000 spores.

TABLE 2. THE EFFECT OF VARIOUS TREATMENTS ON THE PERCENTAGE OF DRY SPORES ADHERING TO THE LOWER EPIDERMIS OF LIVE GRASS LEAVES. (4 leaves per treatment).

Treatment	Paspalum	Cocksfoot	Browntop		Ryegrass	
			Hardened leaves	Soft leaves	Hardened leaves	Soft leaves
A	5	3	2	4	5	4
B	3	—	—	—	1	—
C	—	7	—	—	72	—
D	6	4	3	25	55	66
E	—	—	—	—	35	32
F	101	—	—	—	—	—

— Indicates that no test was carried out.

From the results shown in Table 2, it was concluded that:

(1) Spore adhesion was very low on dry leaves and similar in all species tested.

(2) Where leaves had been previously sprayed with water, most spores were retained by the lower epidermis of ryegrass.

(3) In ryegrass there was little difference between the spore-retaining capacities of hard* and soft* leaves.

(4) Very soft leaves of *Agrostis* were able to retain a large percentage of spores after wetting.

(5) Once spores had settled on a wet leaf and dried on, they were not easily washed off.

(6) As indicated by the detergent treatment on paspalum leaves, the adhesion of spores was largely dependent on the area of the leaf covered by water.

II. *Retention of spores by leaves following application as suspension in water.* The adhesion of spores carried on to dry leaves in water droplets was examined by spraying a suspension of spores in water on to dry leaves.

TABLE 3. THE RETENTION OF SPORES BY LEAVES AFTER THEIR APPLICATION AS A SUSPENSION IN WATER. RESULTS EXPRESSED AS THE AVERAGE OF THE NUMBER OF SPORES RETAINED PER CM^2 OF LEAF SURFACE.
(Six leaves per treatment).

Species	Condition of leaves	Upper epidermis	Lower epidermis
Ryegrass	Wilted	92	867
	Hard	106	1,210
	Soft	88	430
Paspalum	Hard	130	155
	Soft	236	175
Cocksfoot	Hard	63	119
	Soft	219	182

The same spore suspension was applied to each leaf tested. Leaves were disturbed before estimation of spore retention and all larger droplets dislodged as a result. From the counts made (Table 3) it was concluded that:

(a) With ryegrass, spore adhesion was weak on the upper epidermis, but very much stronger on the lower epidemis.

* Hard leaves are defined here as mature leaves in which the cuticle is well developed; soft leaves, conversely, are immature or mature leaves which possess a very thin cuticle.

(b) With paspalum and cocksfoot spore adhesion was weak on both surfaces of the leaves.

(c) There was little difference between the spore retention of the upper epidermis of ryegrass and the upper and lower epidermis of paspalum and cocksfoot. Spore adhesion was weak on all these leaf surfaces.

III. *Wettability of leaf surfaces.* In both these preceding investigations into spore adhesion, it was observed that the number of spores retained was largely dependent on the wettability of the leaf surfaces examined. Accordingly an examination was made of the wettability of the surfaces of the leaf blades of various pasture species (Table 4).

TABLE 4. THE WETTABILITY OF LEAVES EXPRESSED AS THE PERCENTAGE OF THE SURFACE COVERED BY WATER. THE RANGES IN PERCENTAGE OF EACH LEAF WETTED ARE STATED

(Ten leaves per treatment).

Species	Leaf condition	Percentage of leaf surface covered			
		Sprayed and undisturbed		Sprayed and then disturbed	
		Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis
Perennial ryegrass	Hardened leaves grown outside in November	5–30	30–100	0	10–90
	As above, but wilted	20–30	100	0	70–100
	Unhardened foliage grown in warm moist conditions	10–30	95–100	0	95–100
	Soft regrowth from herbage cut 2 days previously	5–30	70–100	0	40–100
	Very hard flag leaves on seed-stalks	5–30	40–100	0	40–90
Paspalum	Leaves grown in warm, moderately dry conditions	5–20	5–15	0	0
Cocksfoot	As for paspalum	5–15	5–15	0	0
White clover	As for paspalum	0–20	20–50	0	40–100

This was judged by estimation of the percentage of the surface covered by visible water. The wettability of the lower epidermis of ryegrass, which is smooth and shiny, was very high, but the wettability of the upper epidermis of ryegrass, and both the upper and the lower epidermis of paspalum and cocksfoot, was low. On the latter, water accumulated as discrete droplets which ran off very easily as soon as the leaf was disturbed. The upper epidermis of white clover had low wettability, but the wettability of its lower epidermis was higher and the spread of water was improved by disturbance.

Conditions affecting germination and subsequent mycelial growth

Although the fungus at no time showed any evidence of becoming parasitic, observations indicated that spores would germinate both on living and on dead leaves. To examine the factors affecting germination on live ryegrass leaves within a pasture, spores were sprayed onto areas of turf carrying mature leaf tissue. Spores were applied in the evening and observations made next morning approximately 15 hours later. On one portion of each area of turf, leaves were left intact, and on another portion the top half of the leaves was cut off immediately before spraying.

TABLE 5. PERCENTAGE GERMINATION OF *P. chartarum* SPORES ON RYEGRASS LEAVES
(Ten leaves per treatment and approximately 100 spores counted per treatment).

Temperature		Near cut ends of leaves				On intact leaves			
		Not germinated	Germinated. Length of germ tube relative to spore length			Not germinated	Germinated. Length of germ tube relative to spore length		
Grass minimum	Grass maximum		0–1	1–5	5+		0–1	1–5	5+
71°F	89°F (g)	28	31	26	15	81	17	2	0
58°F	76°F (g)	41	24	22	13	90	10	0	0
53°F	66°F (g)	72	18	10	0	92	8	0	0
47°F	69°F (n)	97	3	0	0	100	0	0	0

g = plants grown in glass-houses; n = plants grown under natural conditions.

On the intact ryegrass leaves germination was very limited, and spores which did germinate developed no further (Table 5). Germ tubes showed no increase in length between 17 and 41 hours after the spores were applied to the leaves and still had not increased further in length 65 hours after application.

On the other hand, there was rapid germination of spores and vigorous development of mycelium around the cut ends of the ryegrass leaves from which the tops had been removed. Observations along the length of the leaves indicated that the stimulation

of germination and the subsequent growth were related to the area over which the leaf exudate could have been expected to diffuse under the moisture conditions which prevailed. Where the grass minimum temperature fell to 47°F, germination even around cut ends was negligible, and even with a grass minimum temperature of 53°F those spores which had germinated around the cut ends showed restricted development of the germ tubes.

Marked stimulation of spore germination and mycelial growth also occurred around cut ends of leaves of paspalum and cocksfoot.

It was found subsequently that juice pressed out from live ryegrass leaves stimulated germination and mycelial growth at dilutions down to 1–50 or 1–100. Spores put into dilutions in the range from 1–5 to 1–15 produced a visible mycelial mat on a slide in 12–15 hours, and liquid in a Petri dish was gelled in the same time.

Exudates from live leaves

Several investigators (e.g., Tukey *et al.* 1957) have shown that quantities of organic substances and minerals can be moved out through the surface of leaves. As these materials could be potentially available for stimulating germination and mycelial growth, preliminary tests were made to obtain an indication of the quantity of organic material which could be exuded onto the surface of live leaves of ryegrass during wet or moist conditions.

Beds of ryegrass were grown in a warm humid glass-house with a minimum night temperature of 63°F. Some beds received no additional nitrogen source, and with others urea was applied to the soil at a rate equivalent to 4 cwt/acre 3½ days prior to sampling. The immature herbage sampled was all regrowth which had occurred after the commencement of the treatments and had grown

TABLE 6. THE QUANTITY OF NITROGEN PRESENT IN WASHINGS OF RYEGRASS LEAVES EXPRESSED AS PERCENTAGE OF DRY DRY WEIGHT OF HERBAGE

Growth environment	Condition of leaves	Fertiliser treatment	Percentage of nitrogen
Outside	Hardened	None	Nil
Glass-house	Very immature (regrowth from recent cutting—1–2 in. long)	None	0.12
		+ Urea	0.61
Glass-house	Immature (regrowth 2–3 in. long)	None	0.07
		+ Urea	0.46
Glass-house	Moderately mature (4–6 in. long)	None	0.05
		+ Urea	0.34

up above the existing short herbage. For comparison, a sample of hardened ryegrass leaves was taken from herbage which had grown in the field during November.

Samples were cut at approximately 9 a.m., and the leaves were immediately immersed in water for 5 minutes. The water was then drained away and analysed for nitrogen. The samples were not agitated while they were in the water, and no attempt was made to remove any water other than that which freely drained away. The quantity of nitrogen in the water drained off was taken as an indication of the quantity of soluble organic material available at the leaf surfaces (Table 6).

There was no detectable nitrogen in the leaf washings from the hardened herbage grown outside. However, a considerable amount was obtained from the less mature glass-house-grown herbage. The amounts were highest from very soft short regrowth and were increased considerably where urea had been applied to soil a few days previously.

Early development of toxicity

To ascertain whether the very rapid germination and mycelial growth shown by spores in the presence of leaf exudate and leaf juices could be accompanied by parallel rapid increases in toxicity, feeding tests with guinea-pigs were carried out under the supervision of W. A. Te Punga, Wallaceville Animal Research Station, Department of Agriculture. Batches of freshly harvested spores in aqueous suspension were split into two aliquots, one of which was immediately placed in a deep freeze. The other aliquot was placed in a 1–15 dilution of juice pressed from ryegrass leaves and maintained in Petri dishes at approximately 70°F for 15 hours before being deep frozen. All aliquots were kept in a deep freeze until being fed to the test animals. Results of the feeding tests were assessed according to the standards used by Te Punga and MacKinnon (1961). For the quantity of spores used, toxicity was rated 2 for ungerminated spores and rose to 3 for those allowed the 15 hours for germination and growth in the dilute ryegrass juice.

DISCUSSION

The significance of the results reported in this paper must be judged in relation to the factors influencing the development of *P. chartarum* and the occurrence of toxicity in the field.

That the fungus requires warm moist conditions for rapid growth has been well documented (Thornton and Sinclair 1960). Studies of the relation between climatic conditions and the occurrence of facial eczema had earlier suggested that if conditions in the pasture remained too wet, as would result from continued heavy rain and cloudy weather, development of the fungus would be checked (Mitchell *et al.* 1959). The present results are in agreement with those observations. Continuing wetness and the presence of excess moisture in a pasture can be maintained without deep

saturation of the soil below, although thoroughly and deeply wetting the soil will naturally assist in maintaining wetness of the litter in the lower layers of the pasture. Such continued wetness is also more liable to occur on a heavy clay soil, which easily pugs and retains surface moisture, than on a freely-draining permeable soil.

The ability of the fungus to grow rapidly on dead leaves of a wide range of grass species (P. C. Barclay and G. de Latour unpublished) suggests that ryegrass litter should not be regarded as being an exclusive or necessarily a predominant host for the fungus. However, the leaves and stems of perennial ryegrass possess characteristics which are particularly favourable for the maintenance and spread of the fungus in pasture. On the one hand, their toughness provides durability which allows dead leaves and stems to remain nearly intact for long periods, thus maintaining actual or potential sites for growth and survival of the fungus. On the other hand, ryegrass leaves generally have a comparatively high content of nitrogenous and other soluble materials, the presence of which favours a rapid flush of growth of the fungus when conditions become suitable. Such a relation was noted in this work, and was clearly predicted by laboratory studies of the fungal growth requirements (Ross 1960a, b). Previously Thornton and Sinclair (1960) had suggested that pastures might be highly toxic only during periods of active fungal growth and spore production.

Whereas direct ingestion of fungal material from the litter is probably a major source of toxicity for grazing stock, the live leaves of ryegrass do possess a number of characteristics which can render them an important source of toxic material. The lower surface of leaves wets easily and is thereby able to retain a large number of spores. Transference of spores onto live leaves could be induced easily by air movement (Packard 1960) accompanying wind flow or hoof movement, or by droplet splash accompanying rain or treading. Where spores are adjacent to points of damage on a live leaf, such as would come from grazing, mowing, or trampling, they could germinate readily, and for a time grow rapidly. Also flush growth of young leaves can, under warm moist conditions, exude a considerable quantity of organic material, which would probably be of a form stimulating the rapid germination of spores and subsequent growth of mycelium. Flush growth of ryegrass is generally greatest where plants are adjacent to a urine patch or where there is an overall build-up of fertility in the soil, and exudation of organic material could also be expected to be greatest under these conditions.

Contact of spores with this exudate could occur either as a result of their transference onto young leaves or by the downward flow of the exudate towards the base of the tillers and onto the dead sheaths of older leaves. Such sheaths would have been favourably placed to have received spores formed during earlier flushes of fungal growth. Tiller bases of this type would often be taken in by sheep nibbling at the young fresh growth.

The present observations indicate that there can be within a few hours a considerable rise in toxicity from conditions which stimulated prompt germination of spores and rapid proliferation of mycelium.

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REFERENCES

Mitchell, K. J.; Walshe, T. O.; Robertson, N. G. 1959: Weather conditions associated with outbreaks of facial eczema. *N.Z. J. agric. Res. 2:* 584–604.

Packard, R. Q. 1960: The size, shape, and density of spores of *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis. *N.Z. J. Sci. 3:* 412–4.

Percival, J. C.; Thornton, R. H. 1958: Relationship between the presence of fungal spores and a test for hepatotoxic grass. *Nature, Lond. 182:* 1095–6.

Ross, D. J. 1960a: A study of the physiology of *Sporidesmium bakeri* Syd. — 1. Nutrition. *N.Z. J. Sci. 3:* 15–25.

————1960b: A study of the physiology of *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis.—2. Some factors influencing sporulation in culture. *Ibid. 3:* 441–52.

Te Punga, W. A.; MacKinnon, M. M. 1961: Toxicity testing of *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis in guinea-pigs. *N.Z. J. agric. Res. 4:* 147–50.

Thornton, R. H.; Sinclair, D. P. 1960: Some observations on the occurrence of *Sporidesmium bakeri* Syd. and facial eczema disease in the field. *N.Z. J. Sci. 3:* 300–13.

Tukey, H. B.; Wittwer, S. H.; Tukey, H. B. Jr. 1957: Leaching of nutrients from plant foliage as determined by radio-isotopes. *Proc. UNESCO Int. Conf. Radio-isotopes sci. Res. pp.* 1–14.

SOURCES OF VARIATION IN THE WEANING WEIGHT OF ROMNEY MARSH LAMBS

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ABSTRACT

The effects on weaning weight of type of birth and rearing, age of dam, year of birth, sex, and age at weaning were studied on 2,655 records from one flock and 649 records from another flock of Romney Marsh sheep. In the first flock, differences in weaning weight between wether lambs and ewe lambs, lambs from five-year-old and two-year-old dams, and single and twin lambs were, respectively, 3.0 lb, 4.8 lb, and 10.2 lb. The regression on age at weaning was 0.28 lb per day. Effects of the same order were found in the second flock. The differences due to type of birth and rearing were found to be the most important source of variation. The heritability of weaning weight calculated by doubling the regression of offspring on dam was 0.35.

INTRODUCTION

The live weight of a lamb at weaning is a characteristic of economic importance in a dual-purpose breed of sheep such as the Romney Marsh, since it is a convenient measure of the growth of the lamb up to the stage at which weaning occurs. It is also a component of the total weight of lamb weaned by a ewe, a characteristic which Rae (1958a) has suggested as a useful measure of the lamb production of a ewe.

Many factors contribute to the variation in weaning weight of lambs. Factors such as age of the lamb at weaning, its sex, and whether it was born as a twin or a single, tend to obscure genetic differences between lambs. Thus these factors make selection for weaning weight, either alone or as a component of a selection index, less effective. If information on the effects of these factors is available, it can be used to adjust records so that maximum improvement may be achieved. The purpose of this study, therefore, was to determine the effects of type of birth and rearing, age of dam, year of birth, sex, and age at weaning on weaning weight. In addition, an estimate of the amount of genetic variation in weaning weight was obtained.

No information on the sources of variation in the weaning weight of Romney lambs under New Zealand conditions has been published. Studies of environmental effects on weaning weight of sheep have been

made by Hazel and Terrill (1945a, 1946a), Sidwell and Grandstaff (1949), and Blackwell and Henderson (1955). Reports by Hazel and Terrill (1945b, 1946b), Nelson and Venkatachalam (1949), Ragab *et al.* (1953), and Blackwell and Henderson (1955) on the heritability of weaning weight are also pertinent.

EXPERIMENTAL

Source of data

The data used in this study were obtained from two flocks. Flock A, a flock of Romney sheep maintained at Massey College, has been described by Rae (1958b). Features of the management of the flock which are pertinent to the present study are that replacement ewes were selected at random from those available each year, the ewes in each age group were assigned at random to each mating group, and care was taken to ensure that no related groups of animals were given either better or poorer than average conditions of management. Lambs were born between August and October in each year. The date of weaning varied from 13 to 29 December over the period under investigation. Records of the weaning weight of 2,655 lambs born in the years 1948 to 1954 inclusive were used in the analysis of non-genetic variation in weaning weight. In estimating heritability, the records of 1,441 lambs from 482 dams were available.

Additional data were obtained from Flock B, a stud Romney flock located in Southland. Weaning weights of 334 and 315 lambs collected during 1955 and 1956 respectively were used in the study of non-genetic variation in weaning weight.

Method of analysis

The data from Flock A were classified according to year of birth, age of dam (two-, three-, four-, and five-year old), type of birth and rearing (singles, twins, and twins reared singly), and sex of lamb (ewe and wether). In addition, the age in days of the lamb at weaning was included as an independent variable. As there was a marked disproportion in the numbers in the subclasses, the method of fitting constants by least squares as described by Kempthorne (1952) was used. The data from Flock B were analysed in a similar manner. As information on the age of lambs at weaning was not available for 1955, the records from 1955 were analysed separately from those collected in 1956.

The heritability of weaning weight was estimated by doubling the regression of offspring on dam. As there was a variable number of offspring per dam, the method of weighted regression of offspring on dam described by Kempthorne and Tandon (1953) was used. Before this regression was calculated, the records of weaning weights were adjusted for the effects of year, age of dam, type of birth and rearing, sex, and age at weaning, using the estimates from Flock A presented in Table 1.

RESULTS

The means, the standard deviation derived from the error mean squares, and the effects on weaning weight of the various factors are

TABLE 1. ESTIMATES OF ENVIRONMENTAL EFFECTS ON THE WEANING WEIGHT OF ROMNEY LAMBS

	Flock A	Flock B	
	1948–54	1955	1956
Mean (lb)	53.24	54.28	47.81
Standard deviation (lb)	7.75	7.84	8.21
Average age at weaning (days)	104	—	94
Age of dam—			
five-year-old minus two-year-old	4.75	4.09	4.26
five-year-old minus three-year-old	1.98	2.18	0.83
five-year-old minus four-year-old	0.33	2.41	— 0.21
Type of birth and rearing			
single minus twin	10.24	9.99	9.32
single minus twin reared singly	6.68	—	4.37
Sex of lamb			
wether minus ewe	3.05	—	—
ram minus ewe	—	4.82	6.58
Linear regression on age of lamb at weaning	0.28	—	0.17

TABLE 2. ANALYSIS OF VARIANCE OF WEANING WEIGHT

Source of variation	d.f.	Mean squares	Percentage of total variation
Years	6	1,040.46**	2.0
Age of dam	3	2,036.83**	2.1
Type of birth and rearing	2	28,168.13**	45.5
Sex of lamb	1	6,144.55**	3.2
Age at weaning	1	20,157.83**	5.3
Error	2,641	60.14	41.9

**Statistically significant at one per cent level

summarised in Table 1. The deviations from the general mean due to the effects of year of birth were: 1948, 3.01 lb; 1949, 1.45 lb; 1950, 0.83 lb; 1951, —1.36 lb; 1952, —3.38 lb; 1953, —0.31 lb; 1954, —0.24 lb.

Table 2 shows the analysis of variance and tests of significance for the different sources of variation in the data from Flock A. The practical importance of the various factors is also shown in Table 2, where the variation attributed to each source is given as a percentage of the total variation. The first-order interactions between year, age of dam, birth rank, and sex were statistically non-significant.

The heritability of weaning weight from the data from Flock A was 0.35. This estimate was based on 480 degrees of freedom, and had a standard error of 0.048.

DISCUSSION

The effects due to type of birth and rearing were found to be the most important source of variation in weaning weight. Single lambs were approximately 10 lb heavier than twin lambs. This figure is similar to the difference of 9.2 lb found for Rambouillet lambs (Hazel and Terrill 1945a) and 11.7 lb for Columbia, Corriedale, and Targhee lambs (Hazel and Terrill 1946a). Observations on Romney sheep under New Zealand conditions reported by Barnicoat *et al.* (1949) suggest that the greater weight of single lambs at weaning can be explained partly by their higher birth weight and partly by their higher milk intake. Lambs born as twins but reared singly were heavier than twin lambs. This difference is likely to be due largely to the greater amount of milk available to the twin reared singly.

The estimates of the effects due to age of dam from the two flocks showed good agreement. Some of the disadvantage suffered by the lambs from the two-year-old ewes is likely to be due to the lower milk yield of these ewes. Barnicoat *et al.* (*loc. cit.*) reported that the total milk yield of six-year-old ewes for 12 weeks' lactation was 15 per cent higher than that of two-year-old ewes. Similar findings have been reported by Bonsma (1939).

Wether lambs were 3 lb heavier than ewe lambs, while the difference in favour of ram lambs was 4.8 lb and 6.6 lb in the data for two years from Flock B. The difference between ram and ewe lambs in Flock B is smaller than that found by Hazel and Terrill (1945a, 1946a).

Differences between years in Flock A contributed only 2 per cent to the total variation. These differences are of only minor interest because normally comparisons would be made between lambs born in the same year.

The linear regression of weight on age of the lamb at weaning was 0.28 lb per day for Flock A and 0.17 lb for Flock B. No reason can be advanced for this difference, although it is worth noting that the average age at weaning differed by 10 days between the two flocks.

The absence of interaction between the classifications in the present data, a point which was also found by Hazel and Terrill (1945a, 1946a),

indicates that the estimates of the effects are suitable for use as additive correction factors. For example, the effect of birth rank can be minimised by adding 10 lb to the weight of a twin lamb to make it comparable to a single lamb. In this connection the results in Table 2 show that a high proportion of the variation in weaning weight can be eliminated by adjusting for birth rank and age at weaning.

No other estimates of the heritability of weaning weight for New Zealand sheep are available for comparison with present results. In a review of estimates from several breeds in other countries Rae (1956) noted that most of the estimates fell within the range of 0.20 and 0.40.

The weaning weight of the lamb is likely to be affected by the maternal environment supplied by the dam. Thus the ewe influences the weaning weight of her lamb both by the genes she transmits and by the maternal environment she provides. In these circumstances, Koch and Clark (1955) have shown that doubling the regression of offspring on dam estimates the fraction of the variance due to additive gene effects in weaning weight (G) plus the heritable differences in the dam's direct environmental influences (M) plus two-and-a-half times the covariance between the two sources of variation (GM). However the actual response to individual selection for weaning weight is determined by $G + \frac{1}{2}M + 1\frac{1}{2}$ GM. No method is available for estimating these individual components from the present data. Hence it is not possible to state in detail the consequences of selection for weaning weight.

REFERENCES

Barnicoat, C. R.; Logan, A. G.; Grant, A. I. 1949: *J. Agric. Sci. 39:* 44 & 237.

Blackwell, R. L.; Henderson, C. R. 1955: *J. Anim. Sci. 14:* 831.

Bonsma, F. N. 1939: *Univ. Pretoria Pub. Agric. No. 48.*

Hazel, L. N.; Terrill, C E. 1945a: *J. Anim. Sci. 4:* 331.

————1945b: *Ibid. 4:* 347.

————1946a: *Ibid. 5:* 318.

————1946b: *Ibid. 5:* 371.

Kempthorne, O. 1952: "The design and analysis of experiments." p. 91. John Wiley, New York.

Kempthorne, O.; Tandon, O. B. 1953: *Biometrics 9:* 90.

Koch, R. M.; Clark, R. T. 1955: *J. Anim. Sci. 14:* 979.

Nelson, R. H.; Venkatachalan, G. 1949: *Ibid. 8:* 607.

Rae, A. L. 1956: *Advanc. Genet. 8:* 189.

————1958a: *Sheepfmg. Annu. Massey Agric. Coll. 231.*

————1958b: *Ibid. 11.*

Ragab, M. T.; Asker, A. A.; Kadi, M. R. 1953: *Emp. J. Exp. Agric. 21:* 304.

Sidwell, G. M.; Grandstaff, J. O. 1949: *J. Anim. Sci. 8:* 373.

STUDIES OF THE NUTRITIVE VALUE OF NEW ZEALAND DAIRY PASTURES

I. Seasonal changes in some chemical components of pastures

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ABSTRACT

Changes in the proximate constituents of New Zealand dairy pastures were recorded during the major part of the 1958-9 dairying season.

Gross energy values of the herbage dry matter determined by bomb calorimetry during the same period show a 5–6 per cent rise from late spring to early autumn. This increase is associated with a rise in the proportion of clover and in the protein percentage of the herbage. Energy values estimated by applying constant factors to the proximate constituents of the herbage were compared with those determined directly, and the latter values were related to fat, protein, crude fibre, and ash by regression equations. The results are discussed in relation to previously published work and with regard to their application in current pasture-intake studies.

INTRODUCTION

In spite of the importance of pasture to New Zealand's economy, there is a singular lack of published data from which to assess herbage feeding values for various classes of livestock at one particular time, or at different periods of the year. In any programme concerned with improving the efficiency of the livestock industry, it is clearly essential to determine accurately animal feed requirements and the extent to which these can be satisfied by pasture herbage and its conservation products.

Feeding tables currently used in North America and Europe provide the main source of published information on the nutritive values of a wide range of feeding stuffs, and contain some data on the feed values of pasture herbages. The latter have been calculated mainly from the results of chemical analyses and from a limited number of digestibility trials. Much additional information which is specific to New Zealand conditions is required.

It is therefore the purpose of the current series of papers to present the results of long-term pasture feeding studies in progress at

Ruakura Animal Research Station, Hamilton, New Zealand, which have been designed to provide some of these data.

In this first paper, the nature and extent of seasonal changes in both the chemical and botanical compositions of dairy pastures will be described. With regard to the former, it should be recalled that, although the classical methods of analysing feedstuffs have often been criticised as empirical and improvements have been suggested by several workers (Crampton and Maynard 1938; Louw 1941; Crampton and Whiting 1943; Waite and Gorrod 1959), application of these has generally produced little additional information of biological significance but has required much extra work (Lancaster 1943; Ferguson 1948; Richards and Reid 1953). Thus the original Henneberg system, or certain variants of it, remains the best available routine analytical procedure for determining the potential nutritive value of feedstuffs. In the present investigation this system was used for the analysis of pasture samples obtained continuously during the major part of one dairying season.

In addition, the reliability of calculating the gross energy values of feeds by multiplying figures for fat, carbohydrate, and protein by appropriate energy constants has been considered. This is obviously important in assessing the feed values of pastures in terms of total digestible nutrients or gross digestible energy and may be particularly significant under New Zealand conditions, where at different times of the year individual grasses and clovers vary both in quality and yield.

In a second paper, the importance of these results will be considered relative to data obtained from indoor feeding trials on the same pasture herbage.

The pasture requirements of stall-fed dairy cattle for maintenance, bodyweight increase, and milk production during different periods of pasture growth and at various stages of lactation are also under study; the effect of level of intake on the efficiency of pasture utilisation is receiving particular attention.

Ultimately, results obtained indoors must be related to field studies. Accordingly, the large body of data derived from these various metabolism trials are being used to develop methods for estimating the intakes of free grazing animals.

These results will be presented in subsequent papers in this series.

MATERIALS AND METHODS

This experiment extended from the end of October 1958 until the end of April 1959. The herbage was obtained from an area of approximately 35 acres which was being used to supply material for an indoor stall feeding project. Between 8 a.m. and 10 a.m. each day, approximately 2 tons of green material were cut by a forage harvester which had been modified to prevent either bruising or laceration of the grass. Always the object was to cut at the stage when the herbage was in optimum condition for grazing by cattle, i.e., 6 in. –10 in. high for much of the season.

For the first two months the pastures consisted basically of a mixture of perennial rye-grass (*Lolium perenne* L.), short-rotation rye-grass (*Lolium perenne* L. × *Lolium multiflorum* L.) white clover (*Trifolium repens* L.), and varying amounts of Yorkshire fog (*Holcus lanatus* L.) and cocksfoot (*Dactylis glomerata* L.). During summer, clovers, both red and white, assumed dominance over the high-producing grasses, and some fields produced considerable amounts of paspalum. In late summer–early autumn, rye-grass regrowth commenced, but at this stage the proportion of clover was much greater than during the late spring, and these conditions persisted until the end of the trial.

Throughout the trial samples representative of the herbage cut each day were obtained by two independent methods: the first involved field sampling as the cut material was delivered from the forage harvester to the following trailer; the second involved sampling when the material was weighed into the feed boxes. These samples were subsequently processed, stored, and analysed separately. Since invariably there was extremely close agreement between individual analyses, means have been used in presenting the results.

The dry matter percentage of the fresh herbage was determined each day by drying sub-samples for 24 hours in a forced-draught oven at 100°C. Further sub-samples were taken each day for chemical analysis, dried in a forced-draught oven at 40°C and composited for periods of one week. These bulked samples were subsequently ground in a Christy and Norris mill and analysed for total nitrogen, crude fibre, and ether extract by methods recommended by the A.O.A.C. (1955), except that petroleum ether was used for the ether extraction. Gross energy values of the herbage were determined directly by total combustion of a part of the ground samples in a Baird and Tatlock bomb calorimeter. Two pellets, each weighing approximately 1 g and repre-

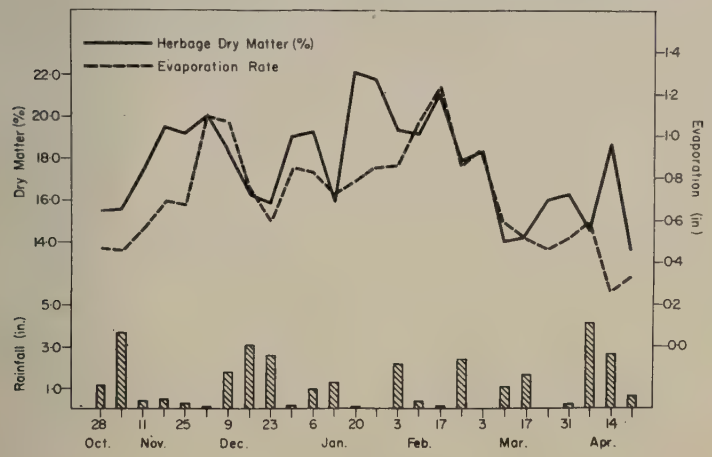

Fig. 1.—Pasture dry matter percentage, total rainfall, and evaporation for individual weeks.

sentative of each bulked sample, were formed in a hand press, dried at 100°c, and burned in 25 atmospheres oxygen. The procedure adopted was essentially as recommended in the Baird and Tatlock handbook. Agreement between duplicate samples was consistently within ± 1 per cent. The ash content was determined from the residues after correcting for unburnt carbon.

RESULTS

The estimated mean dry matter contents of pastures during successive weeks are shown in Fig. 1, together with the total weekly rainfall and water evaporation recorded at Ruakura during the same period. These meteorological data have been included because they help to explain changes in the percentage dry matter of the herbage which are not due to changes in the plants as they pass from the vegetative to the reproductive stage, and in this respect the evaporation rate is particularly useful.

Table 1 shows how the chemical composition of the pasture varied during the 26-week experimental period. There is a strong negative relation between the percentages of crude protein and crude fibre, and a positive relation between those of crude protein and ether extract.

TABLE 1. SEASONAL CHANGES IN PASTURE COMPOSITION
1958–9 SEASON

Period	Percentage composition					Calories/g dry matter	
	Protein (total N × 6.25)	Ether extract	Crude fibre	N.F.E.	Ash	Directly measured	Estimated
22–28 Oct.	15.9	2.2	27.4	48.3	6.2	4,372	4,209
29 Oct.–4 Nov.	16.3	2.4	28.8	46.0	6.5	4,320	4,211
5–11 Nov.	17.8	2.4	25.4	48.1	6.3	4,398	4,245
12–18 Nov.	19.1	2.6	24.4	46.9	7.0	4,390	4,244
19–25 Nov.	18.2	2.7	25.8	47.8	5.5	4,458	4,302
26 Nov.–2 Dec.	14.9	2.5	26.0	51.0	5.6	4,412	4,233
3–9 Dec.	14.8	2.6	25.0	50.7	6.9	4,428	4,183
10–16 Dec.	14.0	2.2	26.7	50.7	6.4	4,353	4,169
17–23 Dec.	15.5	2.1	27.2	49.2	6.0	4,430	4,207
24–30 Dec.	15.5	2.2	29.7	46.9	5.7	4,430	4,223
31 Dec.–6 Jan.	17.2	2.2	26.8	47.9	5.9	4,410	4,241
7–13 Jan.	18.3	2.8	26.4	45.6	6.9	4,440	4,252
14–20 Jan.	16.6	2.1	27.2	47.7	6.4	4,466	4,211
21–27 Jan.	19.5	2.5	23.6	47.8	6.6	4,482	4,267
28 Jan.–3 Feb.	15.2	1.8	28.4	48.0	6.6	4,410	4,160
4–10 Feb.	13.6	1.6	30.6	47.4	6.8	4,363	4,119
11–17 Feb.	18.1	2.4	25.2	47.7	6.6	4,451	4,234
18–24 Feb.	20.6	2.4	21.7	48.4	6.9	4,500	4,265
25 Feb.–3 Mar.	19.9	2.5	22.9	47.2	7.5	4,472	4,238
4–10 Mar.	23.9	2.6	20.6	45.6	7.3	4,486	4,307
11–17 Mar.	25.8	2.7	20.7	43.3	7.5	4,554	4,338
18–24 Mar.	27.0	3.3	19.6	41.8	8.3	4,468	4,357
25–31 Mar.	25.8	3.3	20.2	43.7	7.0	4,562	4,392
1–7 April	23.9	2.7	20.1	46.3	7.0	4,531	4,327
8–14 April	25.4	2.5	17.2	47.5	7.4	4,560	4,325
15–21 April	26.0	3.1	19.7	43.9	7.3	4,548	4,369

The relation between crude protein and crude fibre is more clearly defined than in the work of Woodman *et al.* (1933), Coop and Sears (1942), and Barnicoat (1956), and is in direct contrast with the results of Richards and Reid (1952). Table 1 shows that there was very little change in either the protein or fibre contents until mid-summer, when the protein level fell and that of fibre rose. In the early autumn, the protein fraction rose to a maximum, while the crude fibre showed a corresponding fall.

Seasonal changes in the gross energy value of the herbage are also shown in Table 1. Between October 1958 and April 1959 a range of 230 calories/g was recorded for the mean weekly gross energy of the herbage dry matter. In terms of organic matter this was equivalent to 300 calories/g, and represented a 6 per cent difference between the highest and the lowest values. The slightly greater increase for the organic matter was due to a slight rise in ash percentage.

In calculating total digestible nutrient and gross digestible energy values from results of proximate analyses of feeds and faeces by conventional procedures, it is assumed that the unit weight of fat, carbohydrate, and protein each has a characteristic standard energy value. It is obviously most important that the validity of this approach should be checked before being applied to pasture herbage. In the present investigation the "estimated" calorific values contained in Table 1 have been computed by assuming that 1 g of fat, protein, and carbohydrate contains respectively 9,450, 5,650, and 4,100 calories (Brody 1945), while the "directly measured" values are those obtained by bomb calorimetry.

This comparison shows the estimated values to be consistently lower than those directly determined. One possible explanation is that the analytical methods employed may have failed to separate and measure the fat, carbohydrate, and protein fractions with sufficient

TABLE 2. SUMMARY OF REGRESSION ESTIMATES
(based on deviations about the mean)
Predictand = Calories/g dry matter

Predictor (Mean values in brackets)	Regression coefficients	Intercept	Residual s.d.
Protein % (19.18)	12.95	4,201	37.61
Crude fibre % (24.51)	— 15.53	4,830	37.12
Ether extract % (2.47)	99.43	4,204	53.89
Ash % (6.70)	47.18	4,134	59.85
Protein %	8.77	4,731	34.07
Crude fibre %	— 9.85		
Ash %	— 31.10		

accuracy. The presence of non-ether soluble fats in the carbohydrate fraction, for instance, would cause calorific values to be underestimated, since the conversion factor for carbohydrate is approximately half that of fat. A second possibility is that the standard factors are not valid for pasture extracts.

An attempt was made to test this possibility by partitioning the "total" variance of calories/g herbage dry matter between fat, protein, and carbohydrate, and obtaining standard conversion factors by multiple regression analysis. From a comparable analysis of deviations from the means, it was found that the intercept of the regression on the vertical axis was significantly different from zero (Table 2), so that a set of factors comparable to those currently used could not be derived in this way. Equations based on analyses of deviations from means were obtained, however, and these enabled predictions of calories/g herbage dry matter to be made from the percentages of protein, crude fibre, fat, and ash, both individually and collectively. These are summarised in Table 2. The individual and the three partial regression coefficients are all statistically significant, and the intercepts all differ significantly from zero. In the multiple regression analysis, the partial regression of calories/g dry matter on fat percentage was not significant. The data for fat were therefore excluded from the final analysis. In Table 2 it will be noted also that, whereas the individual regression of calories/g herbage dry matter on ash percentage is positive, the corresponding partial regression is negative. The former is probably due to the slight but steady increase in ash percentage between October and May, the latter to the decline in energy values which occurs at each protein and fibre level as the ash percentage increases. The calorific values of these samples of pasture herbage can be estimated with reasonable accuracy, therefore, by reference in particular to the levels of protein and crude fibre. A small but significant improvement can be obtained also by using both of these predictors, together with the percentage of ash.

DISCUSSION

Very few published data relating to seasonal changes in the chemical composition of New Zealand pastures are available to compare with the present results. Hudson *et al.* (1933) and Coop and Sears (1942) have provided some information on changes in the crude protein, ether extract, crude fibre, and ash fractions of high-producing sheep pastures. Coop *et al.* (1952) have reported on changes in the chemical composition of South Island high-country pastures, and Barnicoat (1956) has analysed samples of herbage from sheep pastures in low and high grass-producing regions of the North Island. In addition to recording changes in components of the ash fraction of herbage from sheep pastures, Sears and Newbold (1942), Sears and Thurston (1952), and Melville and Sears (1953) published data on seasonal trends in total nitrogen, while Johns (1955), using a pure short-rotation rye-grass/white clover sward, has reported changes in total nitrogen, non-protein nitrogen, amino acids, and soluble carbohydrates for a period of 18 months. Only Rigg and Askew (1931) and McIlroy and Bartrum (1940) appear to have published information which relates specifically to New Zealand dairy pastures.

In general, crude protein and fat levels obtained from the present analyses are lower than those reported previously. The reasons may include initial differences in the species' composition of the various pastures; the effect of stage of growth at harvesting, which will differ for sheep and cattle, on subsequent growth and development of different species; and the effect of this on botanical composition. Certain differences may also be associated with variations in the treatment, storage, and analysis of samples at different research stations. In this regard it should be recalled that, whereas in the present investigation well-defined relations were observed between the crude protein, fat, and crude fibre fractions, such relations have not always been shown in previous publications, where the ether extract, especially, has shown marked between-period fluctuations.

The trend in the total nitrogen content of the herbage reported here, which indicated that highest levels were obtained in autumn, intermediate levels in spring and early summer, and lowest levels in mid-summer, though relating to a shorter period, is essentially similar to that described by Melville and Sears (1953) and Johns (1955). Differences mainly involve the months during which lowest and highest nitrogen concentrations were recorded.

With the exception of the current series of results, the only published data on the gross energy values of New Zealand pastures are those of Lambourne (1957). These were derived from sheep pastures at Ruakura Animal Research Station during a 12-month period, and show no seasonal trend. The mean energy value of the herbage as reported by Lambourne (*loc. cit.*) was 4,470 cal/g dry matter, compared with 4,450 cal/g dry matter in the present study. Since no data on chemical composition were presented by Lambourne (*loc. cit.*), it is difficult to explain the relative constancy of the gross energy values determined by him. A preliminary analysis of further data obtained at Ruakura during 1959–60 shows that changes in the gross energy value of pasture herbage similar to those reported in the present study are not peculiar to one particular season. These later results also indicate that the energy content remains high during autumn, winter, and early spring, but falls during the flush of the growing period. It seems probable that the changes in energy values are associated with changes in botanical composition. Between spring, when short-rotation and perennial rye-grasses, Yorkshire fog, and cocksfoot were the dominant species, and mid-summer, when a mixture of clovers, paspalum, rye-grass, and various weed species were present, energy values altered little. They increased markedly in late summer and autumn, however, when clovers became really abundant, and much of the increase seems due to the high protein levels which accompanied this clover dominance.

There seems little doubt that seasonal changes in the energy value of pastures will prove significant in intake studies with grazing animals. In the past, attempts have been made to measure intake in terms of dry matter or organic matter. Some revision of this will be necessary, however, if, as indicated in the present investigation, the nutritive value of feed expressed in either of these forms changes during different periods of the year.

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REFERENCES

A.O.A.C. 1955: "Official methods of analysis of the Association of Official Agricultural Chemists." 8th ed. pp. 368–72. George Banta, Menasha, Wisconsin.

Barnicoat, C. R. 1956: *N.Z. J. Sci. Tech. A38:* 583.

Brody, S. 1945: "Bioenergetics and Growth." p. 30. Reinhold, Baltimore. 1,023 pp.

Coop, I. E.; Sears, P. D. 1942: *N.Z. J. Sci. Tech. A24:* 203.

Coop, I. E.; Darling, M.; Anderson, C. M. 1952: *Ibid. A34:* 507.

Crampton, E. W.; Maynard, L. A. 1938: *J. Nutr. 15:* 383.

Crampton, E. W.; Whiting, F. 1943: *J. Anim. Sci. 2:* 378.

Ferguson, W. S. 1948: *Agric. Progr. 23:* 129.

Hudson, A. W.; Doak, B. W.; McPherson, G. K. 1933: *N.Z. Dep. sci. industr. Res. Bull. 31:* 66–118.

Johns, A. T. 1955: *N.Z. J. Sci. Tech. A37:* 301.

Lambourne, L. J. 1957: *Proc. N.Z. Soc. Anim. Prod. 17:* 63.

Lancaster, R. J. 1943: *N.Z. J. Sci. Tech. A25:* 137.

Louw, J. G. 1941: *Onderstepoort J. vet. Sci. 17:* 165.

McIlroy, R. J.; Bartrum, M. P. 1940: *N.Z. J. Sci. Tech. A22:* 166.

Melville, J.; Sears, P. D. 1953: *Ibid. A35:* 30.

Richards, C. R.; Reid, J. T. 1952: *J. Dairy Sci. 35:* 595.

————1953: *Ibid. 36:* 1006.

Rigg, T.; Askew, H. O. 1931: *N.Z. Dep. sci. Industr. Res. Bull. 26:* 26.

Sears, P. D.; Newbold, R. P. 1942: *N.Z. J. Sci. Tech. A24:* 36.

Sears, P. D.; Thurston, W. G. 1952: *Ibid. A34:* 445.

Waite, R.; Gorrod, A. R. N. 1959: *J. sci. Fd. Agric. 10:* 317.

Woodman, H. E.; Evans, R. E.; Norman, D. B. 1933: *J. agric. Sci. 23:* 419.

EFFECTS OF COW URINE ON GROWTH OF PASTURE AND UPTAKE OF NUTRIENTS

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ABSTRACT

A field trial was conducted on permanent pasture on Marton loam with cow urine and a nearly equivalent rate of KCl. Its objects were:

(1) To determine the apparent recovery of applied nitrogen and potassium.

(2) To evaluate the effect of these treatments on the magnesium and calcium in pasture and soil.

The apparent recovery of applied nitrogen in the herbage was only 10 per cent of that applied in urine. The apparent recovery of potassium in the herbage was 20–23 per cent of that applied in urine or KCl. No difference was observed in the action of potassium whether applied as urine or as KCl.

The effect of urine nitrogen on yields and on N concentration in the pasture lasted only about two months. The effect of applied potassium on yields and K concentration in the pasture was significant for two years.

Urine and KCl depressed the concentration but not the uptake of Mg and P in the herbage and slightly lowered the level of exchangeable magnesium in the soil.

Considerable losses of N and K and some losses of Mg and Ca are likely to occur under urine spots.

INTRODUCTION

Animal excreta are an essential part of the fertility cycle in grazed pastures, as was demonstrated experimentally by Sears (1953). Using sheep, this investigator found that the return of dung and urine to grazed pastures greatly increased yield, especially by the stimulation of growth of perennial ryegrass, and raised the concentration of potassium, phosphorus, and nitrogen in the herbage.

On the other hand, plant nutrients returned in dung and urine are concentrated on relatively small areas. Following the application of urine, Thompson and Coup (1943) found a gradual but appreciable rise in soil nitrate levels. On leaching, this nitrate is expected to take with it an equivalent amount of cations. Thus

in a urine spot the association of nitrate and water-soluble potassic salts might be expected to expose urine potassium, and possibly other ions, such as magnesium and calcium, to a particularly high risk of leaching. The high concentration of potassium alone is likely to lead to accelerated losses of exchangeable magnesium, as shown by Hogg (1960). Saunders and Metson (1959) measured leaching losses of potassium applied as chloride and in the form of urine, in a pot trial. Although conditions in this experiment were somewhat artificial, evidence obtained on accelerated leaching of potassium, calcium, and magnesium pointed to the possibility that similar effects under urine spots or after top-dressing with high rates of KCl might occur in the field.

In the field, however, it is impossible to determine leaching losses without a battery of large lysimeters. For this reason the experiment described below limited itself to a recording of the quantities of nitrogen and potassium applied in urine and to measuring quantitatively the amounts of these elements and of calcium, magnesium, and phosphorus which were taken up by pasture on treated and untreated plots. Measurements of pasture uptake of these elements were carried out until yield, nutrient concentration, and nutrient uptake were equal on treated and untreated plots.

EXPERIMENTAL

Technique

Prelinary trials were carried out by the senior author in 1956 to determine the likely size of urine spots under selected conditions. Water with precipitated chalk added as a marker was poured from a height of 2 ft 6 in.–3 ft on to slightly sloping to level moist ground covered with short dense pasture. The nozzle of the watering can was adjusted to release 2 litres in 10 seconds, or 1½ litres in 7.5 seconds, following the data published by Goodall (1951). On a calm day on nearly flat ground the average area covered was very similar to that found by Petersen *et al.* (1956)—about 400 sq. in.—but less than the 650 sq. in. estimated by Doak (1952). In a strong breeze dispersion of the main stream and much splash resulted in increasing the area to an average of 780 sq. in. On a calm day but on sloping ground the area was increased greatly to over 1,000 sq. in. in individual spots.

Soil

Two experiments were carried out on Marton loam at Marton. This soil type is classified as a Yellow-Grey Earth Transitional to a Yellow Brown Earth (N.Z. Soil Bureau 1954). It is derived from sandy mudstone. There is a compact subsoil which impedes drainage. In the experiments reported drainage was satisfactory owing to mole ploughing. The annual average rainfall is about 40 in.

Experiment 1

(Technique trial)

The first experiment was a technique trial. It was designed:

1. To measure the influence of urine on the concentration of K in the herbage growing just outside the area wetted by urine.
2. To estimate the variability likely to occur in the concentration of K in the herbage on very small plots.

On 13 September 1956 2,000 ml of urine was applied to each of eight plots measuring 14 in. × 30 in. (420 sq. in) (165 lb N and 230 lb K per acre). The pasture consisted almost entirely of white clover, red clover, and perennial ryegrass.

The first sample of herbage was hand cut 7 weeks after the urine was applied; the last sample was taken 7 months later. Some of the results obtained in the first and last cuts are presented in Tables 1 and 2.

TABLE 1. TECHNIQUE TRIAL, 1ST CUT.
% K in herbage dry matter—31 October 1956

Position	Mean of 8 plots	Lowest value	Highest value
Just inside plots	3.51	2.65	4.00
Centre of plots	3.61	3.08	3.96
1– 3 in. outside	3.29	2.40	4.37
3– 5 in. outside	3.05	2.30	3.78
10–13 in. outside	3.02	2.23	3.78

In the interval between cuts the plots were mown and the herbage discarded. Heavy rainfall in May led to flooding of four of the eight plots. These had to be eliminated.

TABLE 2. TECHNIQUE TRIAL, LAST CUT.
% K in herbage dry matter—28 May 1957

Position	Mean of 4 plots		Lowest value		Highest value	
	Grasses	Clovers	Grasses	Clovers	Grasses	Clovers
Inside plots	2.92	1.75	2.18	1.42	4.24	2.40
0– 3 in. outside	2.69	1.64	2.29	1.30	3.67	2.07
3– 6 in. outside	2.54	1.65	2.18	1.14	2.91	2.09
6– 9 in. outside	2.45	1.70	2.19	1.53	2.97	2.09
9–12 in. outside	2.58	1.59	2.18	1.43	3.08	1.84

The results indicate that very little or no transfer of potassium had occurred 3 in. beyond the border of the plots. On the other hand, there appeared to be very high variability in potassium concentration. For this reason the main trial was replicated 10 times and a buffer strip of 5 in. was decided on as being adequate.

Experiment 2

(Main trial)

In the second and main trial the following treatments were put down, replicated 10 times, on 20 November 1957:

1. No treatment. Control.
2. Urine at 2,000 ml to 420 sq. in.
3. KCl at approximately urine equivalent to 420 sq. in.

(A further 5 treatments put down in May 1958 had to be discarded because of the suspected spread of treatments beyond the plots following a very heavy downpour of rain.)

Each individual plot consisted of a "core area," measuring 14 in. × 30 in. (420 sq. in.), surrounded by a 5 in. wide buffer strip. Before applying the urine a rectangle made from 8 in. wide galvanised iron and measuring 14 in. × 30 in. was placed firmly to outline each core area.

KCl was applied dry, and adhering crystals were brushed off the leaves. Urine was applied with watering cans, taking care to avoid any flow of urine beyond the boundary of the core area.

The urine was collected during milking at Flock House, a nearby farm.

Rates of application of nitrogen and potassium, in lb/acre, were as follows:—

Urine		KCl
N	K	K
329	593	518

Herbage was cut, when 3 in.–5 in. high, on the core area and on the 5 in. wide surround, or on an area of 24 in. × 40 in.

A reel mower with 24 in. wide blades was used. Permanent pegs were placed to guide the mower to cut each plot area within ± 1 in. accuracy. Thus the width of the cut buffer strip surrounding the core area varied from 4 in.–6 in., and the plot size cut from 24 in. × 38 in. to 24 in. × 42 in. (Note that treatments were applied to only 14 in. × 30 in.).

The herbage from each plot was weighed, dried, and stored. For chemical analysis separate cuts were analysed at intervals to

determine the potassium status, while the intermediate cuts were bulked. Dissections of pasture species were not carried out.

For soil analyses five 1 in. diameter cores per plot were taken.

RESULTS

(Main trial)

The main trial began satisfactorily; the November 1957 applications did not burn the pasture, and no urine was observed to run outside the core area. Good growth responses to urine, particularly by grass, became apparent within a few weeks; but by the beginning of February, 80 days after application, all plots were dominated by white clover. As judged by eye, clover vigour was best on the KCl-treated plots, followed by those which had received urine. Typical K deficiency symptoms appeared on isolated clover plants on untreated plots. By May 1958 clovers on the "control" plots were small and stunted.

Yields and chemical analyses of herbage quoted refer to the whole area of each plot, measuring 960 sq. in., but urine and KCl were applied only to the 420 sq. in. core of each plot. The area affected by treatments was probably larger than 420 sq. in., and almost certainly smaller than 960 sq. in. The differences in yield and nutrient concentration quoted are therefore smaller than the actual differences on the core area within plots. The calculations of total uptake of nutrients, however, are accurate unless the peripheral effect of treatments extend more than 4–5 in. beyond the boundaries of the treated core.

Yields of pasture herbage

Table 3 presents a summary of herbage yields in lb dry matter per acre, measured on a 960 sq. in. plot basis. In this and the tables following significant differences are determined by Duncan's Test (Duncan 1955).

TABLE 3. SUMMARY OF PASTURE HERBAGE YIELDS
(lb dry matter/acre*)

Treatments	Period			
	20.11.57–14.1.58	14.1.58–19.11.58	19.11.58–23.11.59	23.11.59–12.7.60
Control	1,780 c B	6,520 b B	7,390 b A	2,630 a
Urine	2,850 a A	7,210 a A	7,780 ab A	2,580 a
KCl	1,980 b B	7,110 a A	7,860 a A	2,740 a
Coeff. of variation	8.7%	5.3%	5.6%	10.7%

* Treatments which do not have a common capital letter differ significantly at the 1 per cent level; treatments which do not have a common small letter differ significantly at the 5 per cent level; treatments which have a small letter in common do not differ significantly.

The stimulating effect on yields of urine compared with that of KCl lasted only until the cut of 14.1.58, that is, for about 50 days. Later cuts for which individual yield data are available, although not presented here, show no differences in yield beween the urine and KCl plots.

The relatively small effect of urine nitrogen on yields is no doubt partly due to the time it was applied. The date of application of urine in this trial coincides with a period of the year in which growth responses of grass-clover pasture to nitrogen are usually very small.

In addition, November, December, and January were wet months, with a total rainfall of 17.4 in. The possibility of rapid leaching of nitrogen therefore cannot be excluded.

Growth responses to applied potassium were statistically significant until the end of the second year.

Concentration of nutrients in the pasture herbage

A true measure of treatment effects on the concentration of nutrients in the pasture herbage on the urine-treated area is not possible because of the difference between the area treated (420 sq. in.) and the area cut (960 sq. in.). Also as all herbage of each cut was needed to determine potassium recovery, it was considered impracticable to attempt dissection into grasses and clovers. This limits the interpretation of the data on nutrient concentration in the herbage, since clovers usually have higher concentrations of magnesium, and, especially, calcium, than grasses, and lower levels of potassium.

Except for the initial boost of grass growth on the urine-treated plots the treated core areas appeared to contain a greater proportion of white clover than did control. The percentage concentration of nutrients in the herbage is summarised in Table 4.

At the first cut one month after the trial began, the potassium concentration in the herbage from the untreated plots was near the critical level at which potassium deficiency could be expected in mixed pasture (McNaught 1958).

It is most likely that in the treated core area potassium concentration of the herbage was adequate one year after the trial was laid down but that it fell rapidly to deficiency levels in the first half of the second year of the trial. Under the conditions of this trial, therefore, the application of potassium at the rate of about 9 cwt of muriate of potash per acre protected pasture from which herbage was removed against potassium deficiency for a period of not much longer than one year.

There is little doubt that 18 months after the trial began all treatments were very potassium-deficient and that after two years the effect of treatments on potassium concentrations was negligible.

TABLE 4. CONCENTRATION OF NUTRIENTS IN THE DRY HERBAGE

Treatment	Cut 23.12.57	Cuts 14.1.58–1.4.58	Cut 7.5.58	Cuts 7.7.58–19.11.58	Cuts 6.1.59–2.4.59	Cut 30.4.59	Cuts 9.6.59–23.11.59	Cut 14.12.59	Cuts 23.3.60–12.7.60
1. % K									
1. Control	2.10 c B	1.82 b B	1.50 b B	1.64 b B	1.20 b B	1.12 b B	1.32 b A	1.34 a	1.19 a
2. Urine	3.35 a A	2.79 a A	2.37 a A	2.04 a A	1.45 a A	1.27 ab AB	1.42 ab A	1.30 a	1.22 a
5. KCl	2.92 b A	2.71 a A	2.33 a A	2.15 a A	1.48 a A	1.38 a A	1.48 a A	1.34 a	1.23 a
Coeff. of variation (%)	12.1	8.5	9.4	7.7	11.8	14.1	9.6	5.4	7.1
2. % N									
1. Control	3.63 b B	5.19 a	5.18 a	4.21 a	4.30 b A	4.76 a	3.75 a	4.21 b A	3.83 a
2. Urine	3.91 a A	5.05 a	5.21 a	4.32 a	4.46 a A	4.87 a	3.82 a	4.39 a A	3.88 a
5. KCl	3.68 b AB	5.19 a	5.18 a	4.23 a	4.50 a A	4.91 a	3.83 a	4.37 ab A	3.83 a
Coeff. of variation (%)	5.1	2.9	2.4	3.7	3.4	3.3	3.6	4.0	—
3. % P									
1. Control	0.45 a	0.55 a A	0.43 a	0.44 a	0.41 a A	0.43 a A	0.44 a B	0.49 a A	0.38 a A
2. Urine	0.43 a	0.51 b B	0.42 a	0.42 b	0.38 b B	0.39 b B	0.41 b B	0.43 b B	0.36 ab A
5. KCl	0.44 a	0.52 b AB	0.42 a	0.42 b	0.37 b B	0.39 b B	0.42 b AB	0.44 b B	0.35 b A
Coeff. of variation (%)	6.5	5.3	6.6	3.8	5.6	6.5	3.8	6.4	6.8
4. % Mg									
1. Control	0.28 a A	0.37 a A	0.32 a A	0.28 a A	0.36 a A	0.35 a A	0.29 a	0.33 a	0.37 a
2. Urine	0.25 b B	0.33 b B	0.28 b B	0.25 b B	0.34 ab A	0.32 b B	0.28 a	0.34 a	0.36 a
5. KCl	0.25 b B	0.34 b AB	0.28 b B	0.26 b B	0.33 b A	0.32 b B	0.28 a	0.33 a	0.37 a
Coeff. of variation (%)	5.4	6.1	3.5	5.0	6.2	4.3	7.0	7.2	8.0
5. % Ca									
1. Control	1.12 a A	1.32 a A	1.02 a A	1.02 a	1.12 b A	0.93 a	0.95 a	1.03 a	1.01 a
2. Urine	0.90 c C	1.14 b B	0.96 b A	1.02 a	1.20 a A	0.92 a	0.97 a	1.08 a	0.99 a
5. KCl	1.04 b B	1.27 a A	0.97 b A	1.02 a	1.15 ab A	0.93 a	0.96 a	1.07 a	0.99 a
Coeff. of variation (%)	7.2	5.6	4.2	4.7	7.0	5.1	—	7.6	—

Nitrogen concentration in the herbage

Data on nitrogen concentrations are also presented in Table 4.

There was only a relatively small and fleeting initial effect of urine on levels of nitrogen concentration. This corresponded to the length of period over which yield responses to urine over and above those from KCl were obtained.

Phosphorus concentration in the herbage

Both treatments caused a fairly consistent lowering in the level of phosphorus concentration in the herbage (Table 4). It has been shown in another trial at Marton (During *et al.* 1960) that top-dressing with as little as 1½ cwt KCl per acre each year sometimes has depressed the concentration of P in mixed pasture herbage.

Some effect of the urea and KCl treatment on the lowering of phosphorus concentration in the herbage could therefore be expected while the influence of these treatments on available soil K and on yield were reasonably marked. The depressing effect of the treatments on P concentration persisted, however, to the end of the trial, by which time treatment effects on available soil potassium as measured by plant uptake of this element had disappeared.

Magnesium concentration in the herbage

The urine and KCl treatments consistently depressed the concentration of magnesium in the herbage until winter 1959 (Table 4), that is, until the treatments no longer gave significantly higher potassium levels in the herbage. As clovers are normally higher in magnesium than grasses, this depressing effect could not be due to the changes in species composition observed in this trial.

Calcium concentration in the herbage

McNaught (1959) has shown that high rates of application of potassium may depress the level of calcium in grasses.

The slight depressions of calcium levels in the first six months of the trial are consistent with these results. The additional effect of urine in lowering the level of calcium in the herbage in the early stages of the trial is almost certainly due to changes in species. Grass growth was stimulated by urine, grasses containing much less calcium than clovers.

Effect of treatments on uptake of nutrients

The uptake of N, P, K, Mg, and Ca in the cut herbage was calculated by multiplying dry matter yield by concentration of nutrients on an individual plot basis.

Uptake and recovery of potassium

The uptake data are presented in Table 5.

TABLE 5. THE UPTAKE OF POTASSIUM
(lb K per acre)

Dates of cuts	Control	Urine	KCl	Coeff. of variation (%)
23.12.57 (first cut)	20.9 c C	60.5 a A	32.1 b B	18.0
14.1.58–1.4.58	63.5 b B	110.7 a A	101.6 a A	10.7
7.5.58	8.4 b B	15.7 a A	15.3 a A	21.8
7.7.58–19.11.58	53.5 b B	74.2 a A	77.4 a A	9.6
First year *20.11.57–19.11.58	146.4 c C	261.1 a A	226.3 b B	8.8
6.1.59–2.4.59	47.0 b B	59.4 a A	61.0 a A	14.0
30.4.59	6.9 b B	7.5 ab A	8.8 a A	20.0
9.6.59–23.11.59	37.7 b A	44.2 a A	46.2 a A	15.5
Second year **19.11.58–23.11.59	91.6 b B	111.1 a A	115.9 a A	14.0
14.12.59	4.8 a	5.1 a	5.3 a	14.3
23..3.60–12.7.60	27.1 a	26.8 a	28.8 a	15.3
Total 2 yrs 8 months	269.8 b B	404.1 a A	376.3 a A	9.0
Differences in total uptake from control	—	134.3	106.5	
Potassium supplied	0	593.0	518.0	
% Apparent recovery of supplied K in cut herbage	—	22.6%	20.6%	

* The first year includes all growth from date of laying down (20.11.57) to 19.11.58.

** The second year includes growth from 19.11.58 to 23.11.59; but the first cut measuring this growth was not carried out until 6.1.59.

The treatment effects on the uptake of potassium by pasture were directly related to yields, herbage composition, and concentrations of K in the herbage.

Urine caused a higher uptake of potassium than KCl only for the short period (Table 5) during which it stimulated yields and grass growth to a greater extent than KCl. In fact, the difference in total uptake of K between these two treatments over the whole period was nearly the same as the difference in the first cut.

The recovery of applied potassium in the cut herbage was surprisingly small in spite of quite satisfactory levels of pasture production. Only 21–23 per cent of the applied K were recovered

in the herbage. Even if as much as 10 per cent of the applied potassium had penetrated beyond the 5 in. wide buffer, recovery levels could still be considered low.

Uptake of nitrogen

Over the whole period the pasture on the urine treatments took up about 32 lb/acre more nitrogen than that on the KCl treatment (Table 6). This difference was achieved during the initial short-lived spurt of growth on the urine plots.

Subsequent differences in nitrogen uptake between both treatments and control appear to be caused solely by a better supply of

TABLE 6. UPTAKE OF NITROGEN
(lb N per acre)

Dates of cuts	Control	Urine	KCl	Coeff. of variation (%)
23.12.57 (first cut)	35.8 b B	70.6 a A	40.6 b B	16.1
14.1.58–1.4.58	180.5 b B	200.8 a A	194.5 a AB	7.2
7.5.58	28.9 b A	34.2 a A	33.8 a A	16.2
7.7.58–19.11.58	137.9 b B	157.7 a A	152.6 a AB	8.5
* First year 20.11.57–19.11.58	383.1 c C	463.3 a A	421.3 b B	6.4
6.1.59–2.4.59	169.4 b A	182.8 a A	186.0 a A	8.2
30.4.59	29.3 a	28.7 a	30.8 a	14.7
9.6.59–23.11.59	107.0 b A	118.1 ab A	119.0 a A	10.3
**Second year 19.11.58–23.11.59	305.8 b A	329.7 a A	335.8 a A	7.1
14.12.59	15.1 a	17.7 a	17.2 a	15.3
23.3.60–12.7.60	87.1 a	85.3 a	89.8 a	11.6
Total 2 yrs 8 mths	791.0 b B	895.7 a A	864.1 a A	4.7
Difference in total uptake from control	—	104.7	73.1	
Difference between N uptake; urine—KCl	—	31.6	—	
Nitrogen applied in urine	—	329	—	

* The first year's growth was measured from 20.11.57.

** The second year's growth was measured from 19.11.58. The first cut of this period was on 6.1.59.

potassium, which led to more vigorous growth and higher total yields.

The urine applied nitrogen at the rate of 329 lb/acre but the pasture on the urine plot exceeded the pasture on the KCl plots in uptake of nitrogen by only some 32 lb per acre. As already indicated, November is not a favourable time for nitrogen responses on grass-clover pastures, and the few months following the application of treatments experienced a high rainfall which would encourage leaching.

Doak (1952) has shown that appreciable losses of nitrogen as ammonia may occur in urine spots through ammonification. The urine used was only 1–2 days old and treated with chloroform. It smelt fresh. It is unlikely, therefore, that losses of nitrogen by ammonification occurred before the urine was applied. On the other hand, the wet soil conditions following application could have led to considerable losses by denitrification.

The uptake of phosphorus and magnesium

The greater yield of the urine-treated plots in the first cut led to a slightly higher uptake of phosphorus—7.7 lb P/acre on the urine plots against 4.5 lb and 4.8 lb P/acre on the control and KCl plots respectively. These increases were, statistically, highly significant. Later on slight increases in herbage yield on the treated plots were offset by slight decreases of P concentration in the herbage. This decrease in herbage concentration of P persisted right to the end of the trial when other differences, such as differences in yield and K concentration of the herbage, had disappeared. The total uptake of phosphorus over the 2 years 8 months therefore was not increased by treatments (Table 7).

TABLE 7. UPTAKE OF PHOSPHORUS
(lb P per acre)

Period	Control	Urine	KCl	Coeff. of variation (%)
First year	41.3	45.8	41.9	Not analysed
Second year	31.1	30.5	30.7	Not analysed
Last 8 months	10.4	9.6	10.0	Not analysed
Total 2 yrs 8 mths	82.1 a	86.0 a	82.7 a	5.2

The uptake of magnesium was also significantly higher on the urine plots in the first cut, but thereafter no differences occurred between treatments. As in the case of phosphorus, the uptake of magnesium remained the same because slightly lower magnesium concentrations in the herbage on the treated plots were compensated by slightly higher yields.

The total uptake of magnesium in the pasture for the period of the trial was:

Control—60.8 lb per acre.
Urine— 62.4 lb per acre.
KCl— 61.0 lb per acre.

The small differences between treatments were not statistically significant.

Results of soil analyses

In view of difficulties in accounting for all the potassium applied to soils (e.g., Metson and Hurst 1953; Saunders and Metson 1959), soil analyses were kept to a minimum. Samples for quick tests were taken on four occasions. The first sampling was carried out 80 days after application of treatments, the last sampling 2½ years after application.

The results of the quick test analyses for potassium showed marked differences 80 days after application, fading to very small differences, but still significant 2½ years after application.

Sampling to greater depth than usual, 0–6 in., 6–12 in., 12–18 in., and 18–23 in., was also carried out nearly 2 years after the trial began. Levels of available potassium were slightly higher on the treated plots in the 0–6 in. sample only. There were no differences due to treatments in the levels of pH, calcium, and Truog phosphorus.

Exchangeable cations were determined only once, 2½ years after treatments were applied and shortly before the trial was

TABLE 8. RESULTS OF SOIL ANALYSES
(0 — 3 in.) 16 May 1960

Exchangeable cations (me %)

Treatment	Ca	Mg	K	Na
Control	22.93 a	1.33 a A	0.10 a	0.24 a
Urine	21.80 a	1.18 b B	0.10 a	0.24 a
KCl	21.55 a	1.20 b B	0.10 a	0.24 a
Coeff. of variation (%)	7.8	5.6		

Quick tests

Treatment	pH	Ca	K	P
Control	5.8 a	11.4 a	2.0 b A	7.4 a
Urine	5.8 a	11.1 a	2.3 ab A	6.7 a
KCl	5.8 a	10.6 a	2.5 a A	6.9 a
Coeff. of variation %	2.3	8.4	17.1	—

closed. Results of these analyses, including quick tests, are summarised in Table 8.

Exchangeable potassium was unaffected, while the quick test for potassium still revealed small differences.

A fall of about 0.13 to 0.15 milli equivalent % of exchangeable magnesium resulted from both the urine and the KCl treatments. A fall of similar magnitude was reported by Hogg (1960) as occurring on mown plots of Horotiu sandy loam, Rukuhia, to which most of the cut herbage was returned and which had received 1 cwt KCl per acre each year for five years.

DISCUSSION

The concentration of potassium and nitrogen in cow urine is very variable. The three samples of cow urine used during the course of these experiments were analysed by E. B. Davies (pers. comm.):

Source	% N	% K
Flock House, early spring pasture moist from rain.	0.25	0.35
Flock House, late spring.	0.50	0.90
Ruakura, late autumn and rather dry.	0.93	1.08

A similarly large range of nitrogen values was reported by Doak (1952).

The size of the urine spot may be subject to equally great variations.

These factors alone would allow for a large range of differences in the rate of application of potassium in urine spots. In addition, the properties of the soil type, the time of the year, the type of pasture, and the rainfall incidence during the critical period just before and after urine is deposited will influence the utilisation of urine nitrogen and potassium by pasture and the degree of leaching likely to occur.

In this trial, urine supplying nearly 600 lb K per acre protected this pasture against potassium deficiency for little more than one year. After only 25 months it failed to have a measurable effect on yield and K concentration in the herbage. The apparent recovery in herbage of the potassium applied was only 20–23 per cent. The fate of the potassium not taken up by the pasture has not been determined, but there is indirect evidence that both fixation and leaching were important factors in this trial.

In a laboratory leaching test, using the technique described by Hogg (1960), only 50 per cent of the potassium from an application of 2 cwt KCl per acre to Marton loam were recovered in leachates

or in an exchangeable form in the soil. The balance is presumed to have been fixed. M. Fieldes (pers. comm.) stated that the topsoil of Marton loam contained about 20 per cent of clay vermiculites, of which about 50 per cent were of type A which collapsed on addition of soluble potassium, and the remainder were of type B which collapsed on heating. Both forms can be involved in potassium fixation. Although Agarwal (1960), in reviewing the literature on potassium fixation, reported that most workers considered fixed potassium moderately available to plants, the possibility cannot be excluded that on this soil a large proportion of the potassium applied was fixed by the soil in a form not easily available to pasture plants.

There is also evidence that potassium may be leached from this soil. In a further laboratory experiment using soil from the trial area at Marton, D. E. Hogg (pers. comm.) found that leaching losses of K, applied at the rate of 700 lb per acre in the form of urine, amounted to 22 per cent over a period of 8 weeks. At the time the test was terminated considerable amounts of potassium were still coming through in the leachate.

There is also uncertainty concerning the fate of nitrogen applied in urine. The apparent net recovery was only 10 per cent of the amount applied. A temporary reduction in the intensity of nitrogen fixation by white clover on the urine plots as compared with the plots receiving KCl would decrease the apparent recovery of urine nitrogen. Even allowing for this, however, it is highly probable that there were serious losses of nitrogen, especially by leaching of nitrate and possibly by ammonification and denitrification.

The results suggest that urine is a source of loss of nitrogen in the soil-animal cycle.

Magnesium concentrations were depressed to a similar extent by both urine and KCl. In this trial on a potassium-deficient soil total uptake of magnesium was little affected by urine or KCl because higher herbage yields compensated for lower magnesium concentrations. However, exchangeable magnesium in the soil fell by 0.13 to 0.15 me per cent, or approximately 9 lb magnesium per acre in the top 3 in. of soil.

Some losses of exchangeable calcium probably occurred, but the differences were not statistically significant.

Both urine and KCl consistently depressed the concentration of phosphorus in the pasture.

CONCLUSIONS

Urine may be an important source of loss of potassium and nitrogen and possibly of magnesium. Losses of nitrogen may be made up by legumes but losses of potassium can only be offset by an increased use of potassic fertilisers.

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REFERENCES

AGARWAL, R. R. 1960: *Soils & Fert. 13:* 375–8.

DOAK, B. W. 1952: *J. agric. Sci. 42:* 162–71.

DUNCAN, D. B. 1955: *Biometrics 11:* 1–42.

DURING, C.; LYNCH, P. B.; MCNAUGHT, K. J.; SCOTT, R. S. 1960: *N.Z. J. agric. Res. 3:* 950–93.

GOODALL, V. C. 1951: *Proc. 13th Conf. N.Z. Grasslands Ass.* pp. 86–94.

HOGG, D. E. 1960: *N.Z. J. agric Res. 3:* 377–83.

MCNAUGHT, K. J. 1958: *Ibid. 1:* 148–81.

————1959: *N.Z. J. Agric. 99:* 442.

METSON, A. J.; HURST, FRANCES B. 1953: *N.Z. J. Sci. Tech. 35:* 327–59.

N.Z. SOIL BUREAU 1954: General survey of the soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5:* 74.

PETERSEN, R G.; LUCAS, H. L.; WOODHOUSE, W. W. JR. 1956: *Agron. J. 48:* 440–4.

SAUNDERS, W. M. H.; METSON, A. J. 1959: *N.Z. J. agric. Res. 2:* 1211–31.

SEARS, P. D. 1953: *N.Z. J. Sci. Tech. A35:* 1–29.

THOMPSON, F. B.; COUP, M. R. 1943: *Ibid. A25:* 118–24.

TRAPPING STUDY OF SOME WINGED APHID VECTORS OF PLANT VIRUS DISEASES IN CANTERBURY, NEW ZEALAND

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ABSTRACT

Sticky aphid traps at crop height (26 in.) at each of three sites on the Canterbury Plains—Lincoln (altitude 36 ft above sea level), Darfield (600 ft), and Annat (1,100 ft)—were operated from October 1957 to September 1959.

The most abundant aphid species trapped were *Myzus persicae* (Sulz.), *Aulacorthum solani* (Kltb.), *Brevicoryne brassicae* (L.), *Cavariella aegopodii* (Scop.), *Rhopalosiphum padi* (L.), and *Capitophorus eleagni* (D. Guerc.).

The main period of aphid activity was early summer, when many species were present. Fewer aphid species were flying in autumn, though large flights of some species took place then. At each site there was very little aphid activity in mid-summer, probably as a result of the climatic conditions then prevailing throughout the area.

There were large differences between the three Canterbury regions in the abundance of winged aphids in spring and early summer.

The implications of these findings in relation to plant virus transmission are discussed.

INTRODUCTION

Aphids, by their feeding activities, inflict considerable damage on plants, but they are of even greater economic significance because of their ability to transmit plant virus diseases. In this respect the alatae (winged forms) are more important than the apterae (wingless forms).

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From the economic standpoint, the main plant virus diseases in Canterbury are potato leaf roll, potato virus Y, cauliflower mosaic, turnip mosaic, carrot motley dwarf, and barley yellow dwarf. All of these are transmitted by aphids, the species concerned being *Myzus persicae* (Sulz.), *Aulacorthum solani* (Kltb.), *Macrosiphum euphorbiae* (Thom.), *Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kltb.), *Cavariella aegopodii* (Scop.), and *Rhopalosiphum padi* (L.). It is necessary to know when the alatae of these species are flying and their relative abundance before it is possible to determine their role as virus vectors in the field.

With this in mind, trapping at crop height with sticky traps was commenced in Canterbury in 1957. As cropping is of importance over the whole of the Canterbury Plains (approximately 40 miles wide and rising from sea level to 1,200 ft), three sites, lying east to west across the plains, were selected to cover the altitudinal range.

The results from potato seed certification suggest that the higher parts of the Canterbury Plains are good seed areas, while sea level sites are poor ones so far as virus spread is concerned. Since this could result from lower levels of aphid activity in the higher (western) region, the present project was undertaken to investigate aphid abundance and activity throughout the area. It was soon realised that the whole aphid complex would need elucidation; consequently the scope of the investigation was widened to include the major aphid species.

Trapping has now proceeded for several years. It is proposed to publish the results as they come to hand since information concerning the flight times and abundance of several aphid species is of immediate interest to plant pathologists and entomologists. This paper presents the trapping results for 1957–8 and 1958–9. The results for the latter season were drawn from larger samples and are therefore considered in more detail. It is proposed to await the results of several further seasons of trapping before entering into detailed discussion of their epidemiological significance.

METHODS

The three trapping sites chosen were at Lincoln (36 ft above sea level), Darfield (600 ft), and Annat (1,100 ft). The appropriate grid reference to the N.Z. Provisional 1 mile Map Series (N.Z.M.S.1) are:—

Lincoln—sheet S.83, 867440.
Darfield—sheet S.83, 512574.
Annat—sheet S.74, 430798.

In 1957–8 a single sticky trap (Close 1959) was used at each site. At Lincoln trapping commenced in October 1957 and continued throughout the next 12 months. However, at Darfield and Annat trapping commenced a month earlier and stopped the following April (Annat) or May (Darfield).

TABLE 1. APHIDS ON 1 TRAP AT LINCOLN, 1957–8

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	*Macrosiphum euphorbiae*	Others
4.10.57	1	5					
11.10.57	1	3					
18.10.57	1	6	1		1		
25.10.57	1	2	2		2		
1.11.57	1	13	5	5	7		1
8.11.57	2	8	1	2	9	1	1
14.11.57	6	19	18	98	35	1	12
22.11.57	16	28	54	78	75	2	30
29.11.57	11	37	33	44	84	1	10
6.12.57	16	46	18	15	45		14
13.12.57	1	29	1	5	10		4
20.12.57	5	21	41	3	3		2
27.12.57	1	1	12	2			1
3.1.58			6				2
10.1.58	1		9				
17.1.58			23	1			
24.1.58	1		10				
31.1.58			40				1
7.2.58			23				1
14.2.58	1		47			1	
21.2.58	1		42			1	
28.2.58	9		210		1		
7.3.58	12	1	927				
14.3.58	44		1,139				5
21.3.58	4	1	117				1
28.3.58	2	1	44		1		3
3.4.58	5		97		8		7
10.4.58	2		6				4
17.4.58	2		12				4
24.4.58	1	1	9		6		5
1.5.58	1				3		
8.5.58			9	1	7		2
16.5.58			9	1	3		2
22.5.58			1	1			
29.5.58			3	1	1		1
5.6.58		1	1				1
12.6.58	2		2	1			
19.6.58			1	1			
27.6.58	2	3					
18.7.58	1						
11.9.58	2						2
18.9.58	1						
25.9.58	2	3					
2.10.58	1	4	1	1			
10.10.58	8	17	4	1	2		1
Totals	168	250	2,978	261	303	7	117

In 1958–9 at each trapping site a crop of potatoes was grown adjacent to an experimental crop of brassicas. Three sticky traps were erected at each site on 2 October 1958: one was within the brassica crop, one was between the two crops, and one was within the potato crop. The traps were placed 22 yd apart on an east-west line, the one in the brassicas always being the most easterly.

Trapping was continued throughout 1959, all traps being cleared at weekly intervals where possible.

RESULTS

In this paper results are presented up to the end of September 1959 for all sites.

The main species trapped were *Myzus persicae, Aulacorthum solani, Brevicoryne brassicae, Cavariella aegopodii, Rhopalosiphum padi, Macrosiphum euphorbiae, Lipaphis erysimi, Brachycaudus helichrysi* (Kltb.) and *Capitophorus eleagni.* The numbers of these aphids trapped at each site are summarised in Tables 1–6; information is also given there on the numbers of other aphid species present.

Where blanks occur in the columns, or dates are missing, no aphids were trapped.

TABLE 2. APHIDS ON 1 TRAP AT DARFIELD, 1957–8

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	Others
6.11.57	3	5	5		7	
17.11.57	1	4	3	1	3	
23.11.57	3	3	3	1	6	
1.12.57		6			13	
8.12.57		6	1	2	14	
17.12.57		5	6	1	5	
22.12.57		3	2		6	2
30.12.57	1		4			
5.1.58			1	1		
12.1.58			2	5		
19.1.58						1
27.1.58				2		
3.2.58			9	1		
10.2.58			5		1	
17.2.58			19			1
24.2.58	1		9			
4.3.58			9			
13.3.58			189			
17.3.58	1		15			
31.3.58	1		34			4
7.4.58*						
14.4.58			1			
21.4.58	1					1
29.4.58						1
7.5.58						
Totals	12	32	317	14	55	10

* Missing

TABLE 3. APHIDS ON 1 TRAP AT ANNAT, 1957–8

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	Others
13.11.57	2	6	2		22	1
21.11.57		13	2	10	25	2
28.11.57		3		4	10	
5.12.57		5		1	18	1
13.12.57	1	9	1	2	24	4
22.12.57		1	1	2	15	1
29.12.57		3			1	
5 1.58		5			2	1
16.1.58			2			
28.1.58			5	1	1	
7.2.58			63			
16.2.58		1	34			
23.2.58			55		1	
2.3.58	6		154		4	
9.3.58	3	1	248		1	
16.3.58	5		62		1	
23.3.58	3		75		1	1
30.3.58	1		39			1
7.4.58	1		18			
Totals	22	47	761	20	126	12

Aphids described as "others" include the following species:

Aphis craccivora Koch
A. gossypii Glov.
A. pomi De Geer
Aploneura lentisci (Pass.)
Acyrthosiphon pelargonii group *
Dactynotus sonchi (Geoff.)
Drepanosiphum platanoides (Schr.)
Jacksonia papillata (Theob.)*
Macrosiphum rosae (L.)
Myzus cymbalariae Stroyan *
M. ornatus Laing
Myzaphis rosarum (Kltb.)
Myzocallis castanicola Baker
M. annulatus (Htg.)
Hyperomyzus carduellinus (Theob.)
Ovatus menthae (Wlk).
Rhopalosiphum rufiabdominalis (Sasaki)
Rhopalosiphoninus staphyleae (Koch).

Those species marked with an asterisk (*) appear to be new records for New Zealand. (However, *Myzus cymbalariae* Stroyan was trapped in Auckland by Lamb (1958) but was misidentified as the morphologically similar *M. ascalonicus* Donc.). Information on the relative abundance of the above species can be obtained from the authors, but most records are of occasional individuals only.

TABLE 4. TOTAL APHIDS ON 3 TRAPS AT LINCOLN
Traps erected 2.10.58.

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	*Macrosiphum euphorbiae*	*Lipaphis erysimi*	*Brachycaudus helichrysi*	*Capitophorus eleagni*	Others
10.10.58	39	62	4	4	9	4	1	2		3
16.10.58	8	24	2	3	3			4		1
23.10.58	24	102	4	2	8	2	3	1	1	1
31.10.58	34	124	142	13	20	8	17	10	1	9
6.11.58	49	72	263	179	50	3	26	11		16
13.11.58	48	102	32	67	37	3	12	9	1	10
20.11.58	73	123	179	93	79	10	39	15	2	16
27.11.58	38	74	100	26	57	2	25	11	6	15
4.12.58	17	32	147	20	10	3	5	5	5	7
11.12.58	8	1	72	14	1	2	1	2	2	8
19.12.58		1	14							2
24.12.58	1		18							
31.12.58			19							1
7.1.59			6							
14.1.59			6							
21.1.59			2				1			
28.1.59			36		1					
4.2.59			47		2					
11.2.59	2		150	1		1		1	2	1
18.2.59	2		63		1		4	2	6	1
25.2.59	6		313	1			1	1	5	1
4.3.59	1		648	2	1		2		8	3
11.3.59	6		344		2		4	22	33	4
18.3.59			26		1			4	18	2
25.3.59	3		53	1	1				44	2
1.4.59	2	1	14		4				25	3
8.4.59	4		49		4		1		113	2
15.4.59	5		10	1	6	1	1		76	3
22.4.59	9	1	3	1	4		1		28	4
29.4.59	3	2	1	1	7				43	2
8.5.59	4			1	1		1		6	1
14.5.59	1				1					
27.5.59					1					
3.6.59	1									
1.7.59					1					
22.7.59				1					1	
19.8.59	1									
9.9.59							1			
16.9.59			3							
23.9.59	1		5		1			1		
30.9.59	9		42					1	1	
Totals	399	722	2,817	431	313	39	146	102	427	118

TABLE 5. TOTAL APHIDS ON 3 TRAPS AT DARFIELD
Traps erected 2.10.58.

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	*Macrosiphum euphorbiae*	*Lipaphis erysimi*	*Brachycaudus helichrysi*	*Capitophorus eleagni*	Others
13.10.58	1	7	7	1	1					
21.10.58	4	9	52		1					1
30.10.58	8	27	478	1			1			
6.11.58	25	34	2,382	33	3			2		3
13.11.58	43	74	3,559	33	7		1	1		5
19.11.58	14	31	3,571	74	4				1	2
25.11.58	28	27	3,413	23	5	1	3	2	1	2
3.12.58	18	11	5,669	76	5				1	3
9.12.58	8	6	1,340	5	2	1	1		1	2
18.12.58	2	2	389	3						1
23.12.58		2	31	2						
29.12.58			289	27						1
7.1.59			63	1	1					1
12.1.59			9	1	1					
19.1.59			6		1					
26.1.59			4		1					
3.2.59	1	2	485	5		2				
11.2.59	2	2	481	1						
16.2.59			28							
24.2.59	4		75		2				3	3
2.3.59			20				2		2	
9.3.59	3		687	2			4		2	
16.3.59	8		47		2	1			4	
23.3.59	4	1	7							
31.3.59	1	1	8						1	
6.4.59	5		2		2	2			1	1
13.4.59	8				3	1	1		10	1
20.4.59	1				4					
27.4.59	7				3				4	1
4.5.59	2				1				1	1
11.5.59										
22.6.59	1									
14.9.59	1									
21.9.59	2									
Totals	201	236	23,102	288	49	8	13	5	32	28

TABLE 6. TOTAL APHIDS ON 3 TRAPS AT ANNAT
Traps erected 2.10.58.
(13.10.58, 21.10.58 no aphids trapped)

Date	*Myzus persicae*	*Aulacorthum solani*	*Brevicoryne brassicae*	*Cavariella aegopodii*	*Rhopalosiphum padi*	*Macrosiphum euphorbiae*	*Liipaphis erysimi*	*Brachycaudus helichrysi*	*Capitophorus eleagni*	Others
30.10.58		3								
6.11.58				3						
13.11.58		5	6	38	2		2	5	1	2
19.11.58	1	2	38	16			1		1	3
25.11.58		10	2	52	2			1		9
3.12.58		1	16	16					1	2
9.12.58			16	4	3					
18.12.58		1	4	12	2			2		1
23.12.58			11		1					
29.12.58			7							
7.1.59			18		4					2
12.1.59			56	4	3					
19.1.59	1	1	286	16		2		1		1
26.1.59	1		697	1					1	
3.2.59			400	2					1	1
12.2.59			366	1						
16.2.59	1		194				1			1
25.2.59			321			2				
6.3.59			385					1		1
13.3.59	Record accidentally destroyed									
20.3.59			358							
26.3.59			17		1					
3.4.59			29		1				1	
9.4.59			8		1	2				
16.4.59			1						1	
23.4.59			4							
30.4.59	1	1	4						1	2
8.5.59	2		2		1					1
Totals	7	24	3,246	165	21	6	4	10	8	26

STATISTICAL TREATMENT

The 1958–9 weekly catches were transformed to log (n + 1) for statistical treatment. For total aphids, and for all species analysed, correlations between traps (within sites) were highly significant ($P < 0.01$). The correlation coefficients are tabulated in Table 7.

TABLE 7. CORRELATIONS BETWEEN TRAP CATCHES WITHIN SITES (1958–9)

Correlation coefficients

	Lincoln	Darfield	Annat
All species	.920	.957	.940
B. brassicae	.909	.973	.912
C. aegopodii	.927	.887	.895
M. persicae	.831	.792	
A. solani	.973	.926	
R. padi	.847		
C. eleagni	.895		

Total aphids trapped during each month from October 1958 to May 1959 are shown in Table 8. Where a trapping period overlapped from one month to the next, a calculated proportion of the aphids was placed in each month. Intersite correlations for these totals are given in Table 9.

DISCUSSION AND CONCLUSIONS

Care should be taken in interpreting the results, since the location of trapping sites is always likely to give some bias in favour of species infesting crops adjacent to the area. This is shown in the figures for *Brevicoryne brassicae* for Darfield in the spring of 1958. In this case the traps were adjacent to a heavily infested seed crop of chou moellier. Autumn 1959 figures for the same area are at a level comparable to the other two sites. The deliberate location of traps between potato and brassica crops in this work has given an excellent picture of the relative abundance in time of the species affecting these crops, and has shown some interesting differences between sites. However, the complete absence of a species such as *Aphis gossypii* Glov., which has been heavily trapped in market garden areas only 20 miles from the Lincoln site, when traps were sited adjacent to a pumpkin crop, is an indication of this type of bias. Care should also be taken in interpreting the lower levels of flight incidence recorded for such species as the cereal aphid *Rhopalosiphum padi*. Sufficient specimens have been trapped to give a general picture of the flight pattern, but few conclusions may be drawn about abundance relative to other species, since the trap locations were not particularly related to grasses and cereals which act as hosts of this species. Nevertheless, provided these precautions are borne in mind, a valuable picture can be obtained, from the results, of the distribution over the year and at three sites of alates

TABLE 8. MONTHLY TOTALS OF SOME SPECIES OF ALATE APHIDS TRAPPED FROM OCTOBER TO MAY AT EACH SITE (1958–)

	M. persicae	*A. solani*	*B. brassicae*	*C. aegopodii*	*R. padi*	*C. eleagni*
Lincoln						
October	113	325	197	49	48	2
November	212	382	615	350	224	12
December	14	11	185	22	2	4
January	0	0	69	0	2	0
February	11	0	923	3	2	16
March	11	1	715	2	9	125
April	22	3	63	3	22	264
May	4	0	0	1	1	2
	387	722	2,767	430	310	425
Darfield						
October	18	50	1,066	10	3	0
November	120	169	16,849	215	20	3
December	13	11	3,316	54	3	1
January	1	1	374	5	4	0
February	6	3	726	2	2	5
March	17	2	770	2	2	7
April	22	0	1	0	13	16
May	0	0	0	0	0	0
	197	236	23,102	288	47	32
Annat						
October			0	0		
November			95	124		
December			25	17		
January			1,379	23		
February			1,131	1		
March			611	0		
April			23	0		
May			2	0		
			3,266	165		

of nine important species of aphids. The first seven of these are known to be implicated in the spread of viruses in various farm crops.

The primary purpose of this work was to establish the pattern of the species which may be important as virus vectors. Some differences have been established by various workers in the efficiency of vectors at various seasons, usually related to the period of growth of the host plants. Doncaster and Gregory (1948) found that potato plants are most liable to infection when they are young, i.e., in the early summer

TABLE 9. CORRELATIONS BETWEEN SITES

	Lincoln/Darfield	Lincoln/Annat	Darfield/Annat
M. persicae	.906**		
A. solani	.887**		
B. brassicae	.314	.367	—.262
C. aegopodii	.974**	.965**	.975**
R. padi	.843		
C. eleagni	.962**		
Total	.815*	—.037	—.176

* Significant at 5% level.
** Significant at 1% level.

months. They also found that *M. persicae* played an important part in this spread, while *A. solani* and *M. euphorbiae* (= *M. solanifollii* Ashm.) were insignificant in field transmission. Many other workers have confirmed the conclusion that initial infestation of viruses in potatoes is by spring migrants, and that subsequent spread is within crops or between adjacent crops (Simpson 1940; Broadbent 1953; Day 1955; Bradley and Rideout 1953). Hollings (1955) found high positive correlation between spread of virus Y and leaf roll in potatoes and trap catches of *M. persicae* but not in other species (*M. euphorbiae, A. solani,* or *Aphis rhamni* Fonsc.).

It is possible that similar seasonal considerations may affect virus spread in other farm crops, and for this reason abundance or otherwise of particular species of aphids at certain times or sites may have an important bearing on farm practices such as sowing times, and on aphid-virus control measures.

Tables 4–6 indicate that heavy seasonal migrations of aphids occurred for some species at all sites.

The species may be grouped as follows:—

(a) *Myzus persicae, Aulacorthum solani, Cavariella aegopodii,* and *Rhopalosiphum padi*: alatae most abundant in October and November but with very few trapped in autumn. *M. persicae* and *R. padi* tended to show a lesser peak in autumn.

(b) *Capitophorus eleagni*: very few trapped in early summer but very marked activity in autumn.

(c) *Brevicoryne brassicae*: with two major peaks of abundance, one in early summer and one in autumn. The significance of these results will be discussed elsewhere in relation to static populations of this species.

The results show that there is little aphid activity in midsummer in Canterbury. (Tables 1–6). This is demonstrated well for the month of January at Lincoln. Similar results were found at Darfield, and there was a lesser indication of this effect at Annat in late December. January is usually a hot dry month, when conditions could have a deleterious effect on the host plants and thus on aphid activity. Similar

results were found in Australia by Bald and Norris (1946). This contrasts markedly with results found in Auckland (Lamb 1958), where alate aphids are abundant throughout the summer months. Detailed correlations of these results with climatic data have not yet been attempted.

The presence of large winged populations of the appropriate vectors would enable early transmission of the potato, carrot, and cereal viruses. In the autumn fewer aphids are present and less virus transmission would be expected. However, for the brassica viruses there are large numbers of alate *B. brassicae* present in both early summer and autumn, hence much spread of these viruses could take place in both seasons.

There are large differences between the three Canterbury regions in the abundance of winged aphids in spring and early summer. Considering the total numbers of potato aphids trapped during spring and early summer in 1958 at Annat, Darfield, and Lincoln respectively, the ratio between sites was 1:16:46. For *A. solani* alone the ratio was 1:10:31, while for *M. persicae* the ratio between sites was 1:151:339. The single-trap samples of potato aphids in 1957 also showed much greater abundance at Lincoln but the difference between Darfield and Annat was less marked.

These results indicate that *M. persicae* is virtually absent in November and December and is rare at other times in the Annat area. At Lincoln, on the other hand, *M. persicae* and *A. solani* are most abundant early in the season. This would appear to be the main reason for degeneration of potato seed stocks in that area. Cottier (1931) pointed out that Annat was a good seed potato area, and his survey indicated that *M. persicae* was uncommon there in early summer.

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REFERENCES

Bald, J. G.; Norris, D. O.; Helson, G. A. H. 1946: Transmission of potato virus diseases. 5. Aphid populations, resistance and tolerance of potato varieties to leaf roll. *Aust. Counc. sci. industr. Res. Bull. 196.* 32 pp.

Bradley, R. H. E.; Rideout, D. W. 1953: Comparative transmission of potato virus Y by four aphid species that infest potato. *Canad. J. Zool. 31:* 333–41.

Broadbent, L. 1953: Aphids and virus diseases in potato crops. *Biol. Rev. 28:* 350–80.

Close, R. 1959: Sticky traps for winged aphids. *N.Z. J. agric. Res. 2:* 375–9.

Cottier, W. 1931: An insect survey of potato foliage in New Zealand. *N.Z. J. Sci. Tech. 13:* 125–39.

Day, M. F. 1955: The mechanism of transmission of potato leaf roll virus by aphids. *Aust. J. Biol. Sci. 8:* 498–513.

Doncaster, J. P.; Gregory, P. H. 1948: The spread of virus diseases in the potato crop. *Agr. Res. Counc. Rep. 7:* 189 pp.

Hollings, M. 1955: Aphid movement and virus spread in seed potato areas of England and Wales, 1950–53. *Plant Path. 4:* 73–82.

Lamb, K. P. 1958: Alate aphids trapped in Auckland, New Zealand using Moericke colour traps. *N.Z. J. Sci. 1:* 579–89.

Simpson, G. W. 1940: Aphids and their relation to the field transmission of potato virus diseases in Northeast Maine. *Maine Agric. exp. sta. Bull. 403.* 305 pp.

STUDIES OF THE ECOLOGY OF THE CABBAGE APHID (*Brevicoryne brassicae*) ON BRASSICA FIELD CROPS IN CANTERBURY, NEW ZEALAND

I. Introduction and population study, 1958-9

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ABSTRACT

This paper is the first of a series dealing with field population studies of *Brevicoryne brassicae* on brassica field crops. In 1958–59 field trials at three sites of Calder swede, medium-stemmed chou moellier, and broad-leafed Essex rape were sampled at approximately weekly intervals and records taken of numbers of aphids and plant weights. Flying aphids were also trapped at crop height. Major peaks in aphid numbers on the plants occurred at all sites in late summer and in spring. The former coincided with peak vegetative growth and the latter with flowering and seeding of the biennial host plants. Peak numbers of flying aphids more or less coincided with maximum numbers on the host plants.

Differences in aphid numbers on the various host varieties were not consistent at the different sites. Thus significant differences in aphid numbers were observed between sites and varieties, and in about half the samples the site x variety interaction was highly significant. Over the whole season greatest aphid numbers were found at Annat, and lowest aphid numbers were found on swede plants, irrespective of site.

The spring migration of aphids from seeding plants resulted in the infestation of newly sown crops. The intensity of initial aphid infestation appeared to be largely determined by proximity of seeding crops, but the ultimate intensity of aphid infestation was not necessarily determined by this factor.

Detailed interpretation of these results will be considered in a future paper.

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INTRODUCTION

One of the most urgent requirements in economic entomology is for much more detailed and carefully planned field intelligence on insect pests. As Jacob (1958) pointed out: "It is necessary to know fairly accurately where pests occur, in what order of density and what order of damage they do. Eventually, of course, the vitally important thing is to be able to predict when their numbers are likely to become such as to cause economic damage."

Insect population fluctuations have been widely studied in the laboratory. However, as Milne (1957) has indicated, many difficulties, if not actual fallacies, derive from attempts to apply this information to field conditions. This has led to the launching of several field scale investigations designed to overcome this difficulty (Morris 1960).

The studies reported in this series of papers were planned to supply some basic field ecological information on the cabbage aphid (*Brevicoryne brassicae*), a major pest of brassica crops and an

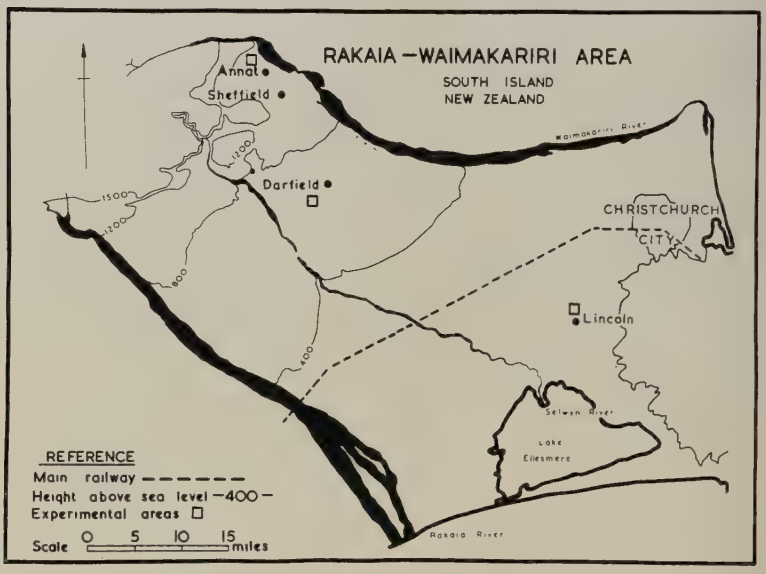

Fig. 1a.—Portion of Canterbury Province, New Zealand, showing location of trial sites (3 squares). (See also Fig. 1b.)

important vector of virus diseases of these crops. Under New Zealand conditions this insect exhibits continuous parthenogenicity, the total population normally consisting of alate or apterous viviparous females. Current farm practice in the area under study supplies a regular succession of cruciferous plants in large blocks—either as seed or fodder crops.

The investigation was confined to certain varieties of field crop brassicas in the Canterbury Province of New Zealand where cabbage aphid damage is severe. Since an intensive rather than an extensive study was indicated and the available manpower was limited, three localities were chosen each season and a replicated field trial was laid down at each site.

In order to obtain some information on the entry of aphids into the crop, the pattern of population growth, and the influence of some environmental factors on these, several types of data were collected. With the aid of sticky traps weekly collections were made of aphids flying at crop height. At weekly intervals throughout the life of the host plants counts were made of alate and apterous cabbage aphids on samples from each plant variety. Plant growth was recorded and also temperature and rainfall at each site.

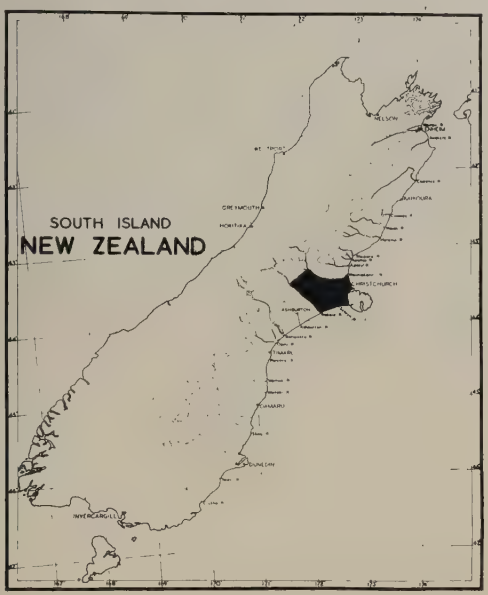

Fig. 1b.—Geographical location of trial sites area shown in Fig. 1a (black area) relative to South Island of New Zealand as a whole.

Since this investigation was planned to extend over several seasons and to include a number of sites and plant varieties, the results of each season's investigations will be reported separately and the combined results discussed later. This paper deals with the results of population studies in the 1958–9 season.

EXPERIMENTAL

TRIAL SITES

Trials were spaced at three sites across the Canterbury Plains, at approximately 20 mile intervals, as shown in Fig 1a. (Fig. 1b shows the geographical location of the trial sites, shown in Fig. 1a, relative to the South Island of New Zealand as a whole.)

Detail of soil was supplied by N.Z. Soil Survey as follows:

Lincoln: soil type. Templeton shallow silt loam, consisting of a greyish brown silt, finely granulated, 9 in. in depth, overlying gravel and stones with a sand matrix. Elevation 50 ft, and annual rainfall averages 25 in. The site is excessively drained.

Darfield: soil type. Templeton silt loam on sandy loam, consisting of 10 in. of brown silt, moderately granular overlying 15 in. of sandy loam on pale yellow loamy sand. Elevation 600 ft, and annual rainfall 32 in. Though freely drained, this soil retains moisture moderately well.

Annat: soil type. Kowai silt loam, being derived from windblown dusts of recent date. It consists of 40 in. of dark brown merging to yellow and olive silt loams, very friable, overlying gravel and stones. Elevation 1,150 ft, and annual rainfall 48 in. There is good drainage, and this soil, being deep and friable, is well suited to cropping.

Climate. All three sites come within Garnier's (1958) Eastern South Island zone. He described this as a homogeneous climatic unit, having external relations with the Antarctic, and characterised by a high variability of rainfall and relatively large mean annual ranges of temperature. All sites are subject to high summer temperatures associated with lengthy periods of low humidity.

PLOT PLANS

Three varieties of field crop brassicas were used in the 1958–9 season:

1. Calder swede.
2. Medium-stemmed chou moellier.
3. Broad-leafed Essex rape.

The first two varieties are normally regarded as aphid-resistant, while broad-leafed Essex rape is known to be highly susceptible to aphid attack (Lowe 1958; Lamb 1960).

A twice replicated Latin square design was used with the same randomisation at each site. Plots were drilled in rows 15 in. apart. At Lincoln plots each comprised 36 rows 30 ft long; at Darfield 16 rows 90 ft long; and at Annat 18 rows 90 ft long. At each site a crop of potatoes was grown adjacent to one side of the trial. Aphid traps were located within the brassica trial, within the potatoes and between the two crops.

Var.	Chou	Rape	Swede	Rape	Chou	Swede
Plot No.	18	13	12	7	6	1
Var.	Rape	Swede	Chou	Swede	Rape	Chou
Plot No.	17	14	11	8	5	2
Var.	Swede	Chou	Rape	Chou	Swede	Rape
Plot No.	16	15	10	9	4	3

Fig. 2.—*Lincoln:* seasonal trends in numbers of *B. brassicae* irrespective of host plant variety and mean number of alate *B. brassicae* trapped per week. (All as log (n + 1)).

METHODS

In the first three samples 10 plants were taken at random from each plot, and the aphids present were counted and classified as apterae or alatae. In subsequent samples five plants were taken from each plot. The individual plants from plots 7, 8, and 9 were weighed in samples 3–14, but in subsequent samplings all plants were weighed. Leaf weights were recorded separately, as well as total plant weights, in samples 33–47.

The technique of aphid trapping used was described by Close (1959).

Weekly aphid counts were transformed to log (n + 1) (Hayman and Lowe 1961) and subjected to analysis of variance within and between sites and varieties. Similar analyses were made on total plant weights and leaf weights both on untransformed data and on log weights.

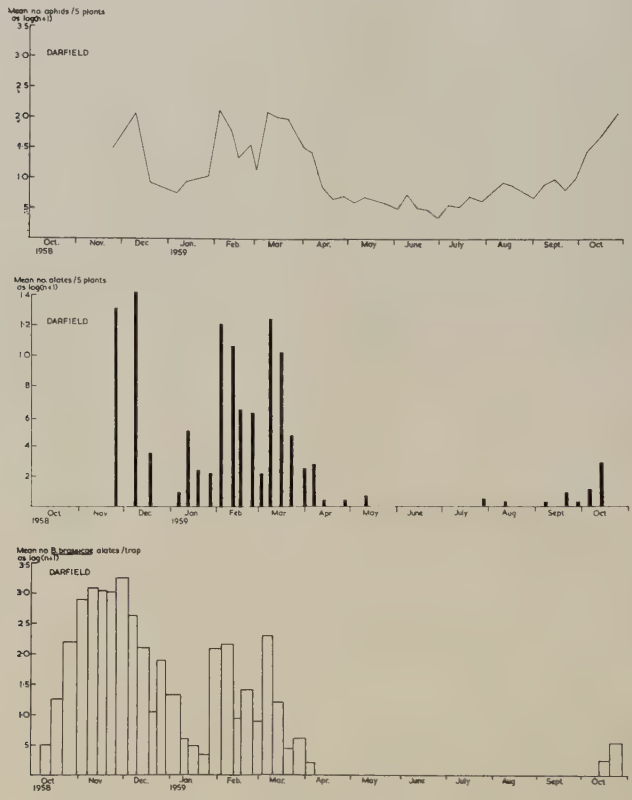

Fig. 3.—*Darfield:* seasonal trends in numbers of *B. brassicae* irrespective of host plant variety and mean number of alate *B. brassicae* trapped per week. (All as log (n + 1)).

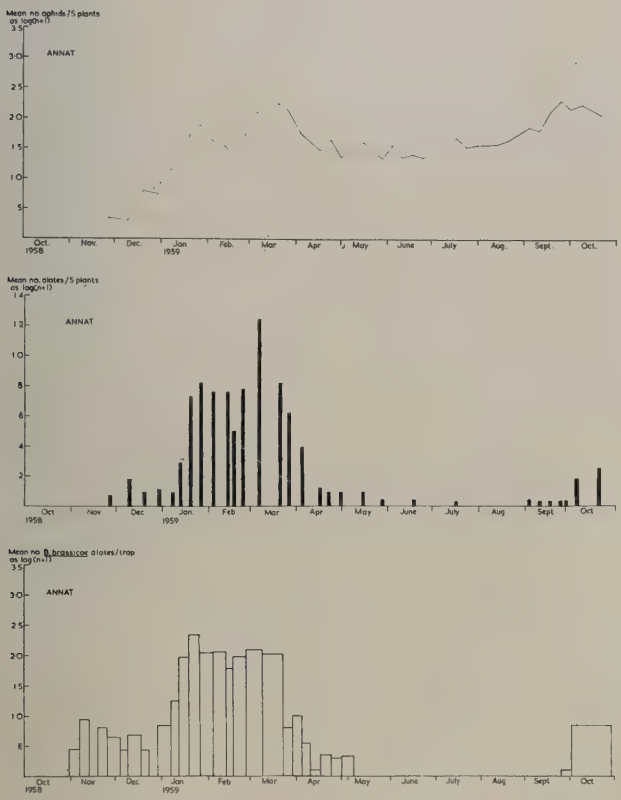

Fig. 4.—*Annat:* seasonal trends in numbers of *B. brassicae* irrespective of host plant variety and mean number of alate *B. brassicae* trapped per week. (All as (log n + 1)).

The aphid counts, trap counts, plant weights, and meteorological data were then transferred to punched cards for further analysis with the aid of a Powers-Samas punched card machine and an IBM-650 electronic computer.

RESULTS

The mean numbers of *B. brassicae* (apterae and alatae) present on each variety of plant are summarised for the separate sites in Tables 1–5.

The seasonal trends in aphid numbers at each site (irrespective of plant variety) are summarised graphically in Figs. 2, 3, and 4.

Text continued on page 637

TABLE 1. MEAN NUMBER OF APTEROUS *B. brassicae* PER 5 PLANTS AS LOG (n + 1) AT LINCOLN

Date	Sample number	Days from sowing	Swede	Chou moellier	Rape	Significant difference between varieties*
13/11/58	1	20	0	.08	.08	—
24/11/58	2	31	.48	.51	.05	—
10/12/58	3	47	.14	.28	.76	+
19/12/58	4	56	.10	.28	.91	++
30/12/58	5	67	0	.38	.40	—
7/1/59	6	75	0	0	.12	
13/1/59	7	81	0	.39	.05	+
20/1/59	8	88	.13	.47	.58	—
27/1/59	9	95	0	.41	.18	+
2/2/59	10	101	.26	.59	.31	—
9/2/59	11	108	.18	.70	.77	—
17/2/59	12	116	.46	.85	1.17	++
24/2/59	13	123	.15	.80	1.16	—
3/3/59	14	130	.88	1.54	1.81	++
11/3/59	15	138	2.13	2.03	2.53	+
18/3/59	16	145	1.80	2.03	2.51	++
25/3/59	17	152	1.55	2.65	2.99	+++
1/4/59	18	159	1.55	2.26	2.80	+++
8/4/59	19	166	1.13	2.23	2.73	+++
15/4/59	20	173	.91	1.57	2.63	+++
22/4/59	21	180	.41	1.23	2.29	+++
29/4/59	22	187	.59	1.56	1.61	+
—	23	—	—	—	—	
14/5/59	24	202	.15	1.36	.73	+++
27/5/59	25	215	.08	.98	.36	++
3/6/59	26	222	.10	.88	.41	+
10/6/59	27	229	.13	.88	.40	++
17/6/59	28	236	0	.91	.55	+
24/6/59	29	243	.15	1.11	.75	—
1/7/59	30	250	.34	.87	.87	++
8/7/59	31	257	.28	1.03	.91	+
15/7/59	32	264	.13	.88	.99	+
22/7/59	33	271	0	.94	.90	—
29/7/59	34	278	.26	.63	.68	—
12/8/59	35	292	.44	1.21	.95	—
19/8/59	36	299	.35	1.00	.55	—
2/9/59	37	313	.72	1.56	1.51	—
9/9/59	38	320	.49	1.63	1.15	—
16/9/59	39	327	1.20	2.15	2.23	—
23/9/59	40	334	1.40	2.25	2.17	—
30/9/59	41	341	1.69	2.10	3.31	++
7/10/59	42	348	1.92	2.65	3.30	++
16/10/59	43	357	2.72	2.85	3.62	+

* The following key applies to Tables 1–8:—

— = N.S.
\+ = P 0.05
++ = P 0.01
+++ = P 0.001

TABLE 2. MEAN NUMBER OF APTEROUS *B. brassicae* PER 5 PLANTS AS LOG (n + 1) AT DARFIELD

Date	Sample number	Days from sowing	Swede	Chou moellier	Rape	Significant difference between varieties
25/11/58	2	25	1.00	1.13	1.32	—
9/12/58	3	39	1.61	2.04	2.18	++
19/12/58	4	49	.79	1.06	.87	—
—	5	—	—	—	—	
6/1/59	6	67	.60	.57	1.07	+
12/1/59	7	73	.97	1.05	.61	—
19/1/59	8	80	.75	1.10	1.02	—
27/1/59	9	88	.76	1.22	1.06	—
3/2/59	10	95	1.99	2.28	1.95	—
11/2/59	11	103	1.52	1.97	1.79	—
16/2/59	12	108	1.19	2.01	.79	+
24/2/59	13	116	1.19	1.73	1.61	—
2/3/59	14	122	.88	1.38	1.06	—
9/3/59	15	129	2.06	2.24	1.82	—
16/3/59	16	136	1.57	2.31	2.03	+
23/3/59	17	143	1.56	2.35	1.95	++
31/3/59	18	151	1.12	1.80	1.56	—
6/4/59	19	157	.58	2.01	1.22	++
13/4/59	20	164	.52	1.24	.79	—
20/4/59	21	171	.30	1.19	.48	+
27/4/59	22	178	.62	.96	.53	—
4/5/59	23	185	.20	.67	.89	—
14/5/59	24	195	.15	.81	1.07	+
25/5/59	25	206	.23	.90	.58	+
2/6/59	26	214	.19	.98	.30	+
8/6/59	27	220	.52	.92	.74	—
15/6/59	28	227	.20	.74	.59	—
22/6/59	29	234	.15	.87	.38	+
29/6/59	30	241	.21	.59	.22	—
6/7/59	31	248	.25	1.00	.43	—
13/7/59	32	255	.25	.81	.50	—
20/7/59	33	262	.27	1.07	.74	+
28/7/59	34	270	.15	.82	.89	—
11/8/59	35	284	.73	1.36	.70	—
17/8/59	36	290	.42	1.15	1.04	—
31/8/59	37	304	.24	.67	1.14	—
7/9/59	38	311	.05	1.72	.91	+++
14/9/59	39	318	.47	1.07	1.41	—
21/9/59	40	325	.10	1.56	.67	+
28/9/59	41	332	.05	1.58	1.41	++
5/10/59	42	339	.66	2.08	1.54	+
12/10/59	43	346	.39	2.18	2.33	+++

TABLE 3. MEAN NUMBER OF APTEROUS *B. brassicae* PER 5 PLANTS AS LOG (n + 1) AT ANNAT

Date	Sample number	Days from sowing	Swede	Chou moellier	Rape	Significant difference between varieties
26/11/58	2	27	.37	.08	.37	—
9/12/58	3	40	.10	.20	.52	—
19/12/58	4	50	.66	.59	1.06	—
29/12/58	5	60	.67	.75	.76	—
7/1/59	6	69	.71	1.33	1.37	—
12/1/59	7	74	.60	1.43	1.95	+
19/1/59	8	81	.92	1.49	2.54	++
26/1/59	9	88	1.05	1.76	2.72	++
3/2/59	10	96	.54	1.71	2.49	+++
12/2/59	11	105	.40	1.77	2.33	++
16/2/59	12	109	.49	1.20	2.45	++
25/2/59	13	118	.87	1.41	2.83	++
6/3/59	14	127	1.39	1.60	3.17	++
—	15	—	—	—	—	
20/3/59	16	141	1.20	2.23	3.22	+++
26/3/59	17	147	1.33	2.10	2.99	+++
3/4/59	18	155	1.11	1.56	2.58	+++
—	19	—	—	—	—	
16/4/59	20	168	.78	1.47	2.18	+++
23/4/59	21	175	1.03	1.68	2.15	+++
30/4/59	22	182	.62	1.52	2.05	++
—	23	—	—	—	—	
15/5/59	24	197	.87	1.85	2.09	+++
28/5/59	25	210	.68	1.74	1.54	++
4/6/59	26	217	.94	1.85	1.84	++
11/6/59	27	224	.70	1.65	1.67	+++
18/6/59	28	231	.94	1.66	1.59	+
25/6/59	29	238	.58	1.68	1.84	+
2/7/59	30	245	.82	1.63	1.88	+++
—	31	—	—	—	—	
16/7/59	32	259	1.08	1.92	1.99	++
23/7/59	33	266	.83	1.65	2.07	+
30/7/59	34	273	.80	1.88	2.00	++
13/8/59	35	287	.88	2.16	1.68	++
20/8/59	36	294	.75	1.97	2.17	—
3/9/59	37	308	.99	2.11	2.48	+++
10/9/59	38	315	1.10	1.98	2.31	++
17/9/59	39	322	1.69	2.30	2.39	—
24/9/59	40	329	1.68	2.40	2.80	+++
1/10/59	41	336	1.62	2.22	2.66	+++
8/10/59	42	343	1.72	2.24	2.72	+
22/10/59	43	357	1.94	1.99	2.46	++

TABLE 4. APTEROUS *B. brassicae* PER 5 PLANTS AS LOG (n + 1) WEEKLY MEANS FOR SITES AND VARIETIES

Sample number	Mean log (n + 1) apterae / 5 plants			All sites			Significant difference		
	Lincoln	Darfield	Annat	Swede	Chou moellier	Rape	Between sites (P)	Between varieties (V)	Interaction (PV)
1	.05	—	—						
2	.35	1.15	.27	.62	.57	.58	+++	—	—
3	.39	1.95	.27	.62	.84	1.15	+++	+++	—
4	.43	.91	.77	.51	.64	.94	++	—	—
5	.26	—	.72	.33	.56	.58	+	—	—
6	.04	.75	1.14	.44	.63	.85	+	+	—
7	.15	.88	1.32	.52	.96	.87	+	—	++
8	.39	.96	1.66	.60	1.02	1.38	+++	+++	+
9	.20	1.01	1.84	.60	1.15	1.32	+++	+++	+
10	.39	2.08	1.58	.93	1.53	1.58	+++	+++	+++
11	.55	1.76	1.51	.70	1.48	1.64	+++	+++	+
12	.82	1.33	1.38	.71	1.35	1.47	+++	++	+++
13	.70	1.51	1.70	.74	1.31	1.86	+++	+++	+
14	1.41	1.10	2.06	1.05	1.51	2.01	+++	+++	+
15	2.22	2.04	—	2.09	2.14	2.17	—	—	+
16	2.11	1.97	2.22	1.52	2.19	2.58	+++	—	+++
17	2.40	1.96	2.15	1.48	2.37	2.64	+++	++	+++
18	2.20	1.49	1.75	1.26	1.87	2.32	+++	+++	++
19	2.03	1.27	—	.85	2.12	1.98	+++	+++	+++
20	1.70	.85	1.48	.74	1.43	1.86	+++	+++	++
21	1.31	.66	1.62	.58	1.37	1.64	+++	+++	+++
22	1.25	.70	1.40	.61	1.35	1.40	+++	++	+
23	—	.59	—	(.20)	(.67)	(.89)			
24	.75	.68	1.60	.39	1.34	1.30	+++	+++	+
25	.47	.57	1.32	.33	1.21	.83	+++	+++	—
26	.46	.49	1.54	.41	1.23	.85	+++	+++	—
27	.47	.73	1.34	.45	1.15	.94	+++	+++	+
28	.48	.51	1.40	.38	1.10	.91	+++	+++	—
29	.67	.47	1.36	.29	1.22	.99	+++	+++	—
30	.69	.34	1.44	.46	1.03	.99	++	+	+++
31	.74	.56	—	.26	1.02	.67	++	—	—
32	.67	.52	1.67	.49	1.20	1.16	+++	+++	—
33	.61	.69	1.52	.37	1.22	1.24	+++	+++	—
34	.52	.62	1.55	.40	1.11	1.19	+++	+++	—
35	.87	.90	1.57	.68	1.57	1.11	+++	++	—
36	.63	.87	1.63	.51	1.37	1.25	++	+++	—
37	1.26	.68	1.86	.65	1.45	1.71	+++	+++	—
38	1.09	.89	1.80	.54	1.78	1.46	+++	+++	—
39	1.86	.98	2.12	1.12	1.84	2.01	++	+++	—
40	1.94	.78	2.29	1.06	2.07	1.88	+++	+++	—
41	2.37	1.01	2.17	1.21	1.96	2.46	+++	+++	+
42	2.62	1.43	2.32	1.44	2.32	2.52	+++	+++	+
43	3.07	1.64	2.13	1.68	2.34	2.81	+++	+++	++

TABLE 5. MEAN NUMBER OF ALATE *B. brassicae* PER 5 PLANTS
(Significance test based on values transformed to $\sqrt{n + \frac{1}{2}}$)

Sample number	LINCOLN					DARFIELD					ANNAT				
	Swede	Chou moellier	Rape	Mean	Significant difference between varieties	Swede	Chou moellier	Rape	Mean	Significant difference between varieties	Swede	Chou moellier	Rape	Mean	Significant difference between varieties
1	.17	0	0	.06											
2	.17	.17	0	.11		14.2	12.5	31.0	19.2	+	.33	.17	0	.17	
3	33	.67	1.17	.72	—	18.2	31.3	25.7	25.1		0	.50	.83	.50	
4	.17	0	.17	.11		.33	2.83	.50	1.22	—	.50	.17	0	.22	
5	0	0	0	0							.33	.33	.17	.28	
6	0	.17	0	.06		.17	0	.50	.22		0	.33	.33	.22	
7	0	0	.17	.06		1.33	3.83	1.33	2.17	—	.83	.83	1.17	.94	—
8	0	0	.17	.06		.67	1.00	.50	.72	—	1.33	6.83	4.83	4.33	—
9	0	0	0	0		.33	1.17	.50	.67	—	1.00	2.17	13.50	5.55	+++
10	.17	.17	.50	.28		5.33	32.50	7.33	15.05	—	1.17	3.50	9.67	4.78	+
11	0	.17	.17	.11		9.83	19.00	9.17	12.67	—	.33	5.50	8.33	4.72	—
12	.50	.33	1.33	.72	—	1.33	7.67	0	4.15	+++	0	1.33	5.17	2.17	—
13	.17	.50	1.33	.67	—	3.17	3.50	3.83	3.50	—	1.17	5.33	8.50	5.00	+
14	10.50	12.17	27.50	16.74	+	.33	1.17	.67	.72	—	5.33	9.17	34.17	16.22	++
15	10.83	16.33	30.33	19.18	—	18.67	21.17	16.83	18.90	—					
16	4.67	3.83	6.33	4.95	—	4.83	18.50	6.67	10.00	++	1.50	6.17	9.00	5.55	—
17	.83	2.33	2.50	1.89	—	1.17	6.00	1.17	2.78	++	.17	2.00	7.33	3.17	+++
18	.33	1.17	2.83	1.44	++	1.00	1.17	.17	.78	—	.33	1.33	2.67	1.44	+
19	.67	2.00	4.00	2.22	+	.17	2.17	.33	.89	—					
20	0	.17	.17	.11	—	0	.17	0	.06		0	.17	.83	.33	—
21	0	0	.17	.06		0	0	0	0		.17	.50	0	.22	
22	0	0	0	0		0	0	.17	.06		.17	.33	.17	.22	
23						0	0	0	0						
24	0	0	0	0		0	0	.5	.02		0	.50	.17	.22	

| | | |

TABLE 6. MEAN PLANT WEIGHT (g) : LINCOLN
(Significance tests based on values transformed to log n)

Sample number	Total plant weight				Leaf weight			
	Swede	Chou moellier	Rape	Sig. diff. between varieties	Swede	Chou moellier	Rape	Sig. diff.
3	1.4	1.0	4.2					
4	8.0	6.6	24.4					
5	2.3	4.9	2.9					
6	0.1	2.6	4.8					
7	12.0	3.2	6.4					
8	5.3	8.2	5.3					
9	3.3	3.9	1.6					
10	6.1	5.0	1.4					
11	7.0	4.2	8.4					
12	3.4	6.2	4.6					
13	10.8	6.6	6.4					
14	8.8	9.9	13.2					
15	14.7	14.9	26.9	++				
16	40	23	62	+				
17	34	36	63	+				
18	56	42	87	+				
19	65	36	75	—				
20	57	58	82	—				
21	56	81	77	—				
22	53	71	98	—				
23	—	—	—	—				
24	72	51	73	++				
25	98	85	51	—				
26	49	45	50	—				
27	75	58	50	—				
28	67	41	65	—				
29	89	75	48	+				
30	70	50	64	+++				
31	89	95	66	—				
32	72	64	49	—				
33	93	59	47	—	18	17	13	—
34	63	61	46	—	11	17	15	—
35	63	62	41	+	13	18	11	+
36	75	107	39	+++	18	29	11	+++
37	116	116	54	+	26	31	21	—
38	62	117	35	+++	18	31	12	++
39	93	92	50	+	24	20	17	—
40	131	106	48	++	23	23	15	—
41	88	133	64	++	17	27	20	+
42	71	65	40	++	13	13	9	++
43	80	114	29	++	13	16	8	+

TABLE 7. MEAN PLANT WEIGHT (g): DARFIELD

Sample number	Total plant weight				Leaf weight			
	Swede	Chou moellier	Rape	Sig. diff. between varieties	Swede	Chou moellier	Rape	Sig. diff.
3	2.6	2.0	3.6					
4	4.7	4.8	8.8					
5	—	—	—					
6	18.8	11.6	35.9					
7	29	36	12					
8	18	66	31					
9	24	46	7					
10	47	20	20					
11	11	53	17					
12	30	49	15					
13	117	115	28					
14	79	25	24					
15	71	71	31	—				
16	85	91	53	+				
17	109	91	53	—				
18	107	91	43	++				
19	138	226	131	+				
20	173	163	83	+				
21	149	185	53	+				
22	134	134	54	+++				
23	126	180	63	+				
24	141	103	68	—				
25	168	183	39	+++				
26	130	189	50	+++				
27	210	182	55	+++				
28	139	193	73	+				
29	136	187	51	++				
30	161	167	35	+++				
31	125	234	55	+++				
32	173	168	41	+++				
33	128	185	43	+++	15	73	9	+++
34	138	167	90	+	18	57	19	—
35	—	—	—		—	—	—	
36	136	178	49	++	17	78	16	+++
37	97	178	44	+	16	66	15	++
38	95	270	42	+++	20	97	10	+++
39	110	218	56	++	33	85	17	+++
40	160	220	49	+++	32	78	20	+
41	121	180	79	++	24	56	29	+
42	152	295	70	+++	26	94	25	++
43	152	300	69	+	33	100	20	++

TABLE 8. MEAN PLANT WEIGHT (g): ANNAT

	Total plant weight				Leaf weight			
Sample number	Swede	Chou moellier	Rape	Sig. diff. between varieties	Swede	Chou moellier	Rape	Sig. diff.
3	3.6	1.4	9.7					
4	—	—	—					
5	38	14	23					
6	86	20	70					
7	25	43	123					
8	165	63	71					
9	83	69	97					
10	139	58	127					
11	59	51	94					
12	111	103	46					
13	92	51	66					
14	42	62	62					
15	—	—	—					
16	87	102	79	—				
17	96	116	93	—				
18	99	83	74	—				
19	—	—	—					
20	129	109	87	—				
21	132	132	125	—				
22	72	150	82	—				
23	—	—	—					
24	138	145	108	—				
25	115	178	59	—				
26	143	124	104	—				
27	114	108	56	+				
28	139	193	73	+				
29	93	109	85	—				
30	101	98	86	—				
31	—	—	—					
32	69	167	71	—				
33	81	76	80	—	4	23	10	—
34	71	90	68	—	4	17	11	+++
35	64	136	38	++	4	25	6	+++
36	49	122	71	+	4	20	13	++
37	45	102	79	+	6	25	14	++
38	42	83	73	—	8	15	15	++
39	58	124	60	—	6	26	13	+
40	58	141	79	+	7	28	14	+++
41	69	101	89	—	8	18	16	—
42	53	106	103	—	7	24	22	+
43	66	99	58	—	7	17	9	—

TABLE 9. ANALYSIS OF VARIANCE OF MEAN LOG (n + 1) APTEROUS APHIDS PER FIVE PLANTS PER MONTH FOR ALL SITES AND HOST VARIETIES

Source of variation	df	MS	F
Varieties (V)	2	5.681066	106.40***
Sites (S)	2	2.854252	53.46***
Months (M)	11	2.474481	46.34***
V × S	4	.384934	7.21***
V × M	22	.108991	2.04*
S × M	22	.542640	10.16***
V × S × M	44	.0533930	
Total	107		
Varieties			
(a) Swedes vs. Chou moellier and rape	1	11.216691	210.08***
(b) Chou vs. Rape	1	.145441	2.72 (NS)
Varieties × Sites			
(a) × Sites	2	.296678	5.56**
(b) × Sites	2	.473188	8.86***
Sites			
Annat vs. Lincoln and Darfield	1	5.689784	106.56***
(b) Chou moellier vs. Rape	1	.018721	(N.S.)

TABLE OF MEANS

	Lincoln	Darfield	Annat	Overall mean
Swede	.612	.643	.899	.718
Chou moellier	1.198	1.264	1.609	1.357
Rape	1.273	1.080	1.987	1.447
Overall mean	1.028	.996	1.499	1.174

* = P 0.05
** = P 0.01
*** = P 0.001
N.S. = Not significant

TABLE 10. ANALYSIS OF VARIANCE OF MEAN LOG (n + 1) APTEROUS APHIDS PER FIVE PLANTS PER MONTH FOR JUNE-AUGUST 1959

Source of variation	df	MS	F
Varieties (V)	2	1.535263	188.32***
Sites (S)	2	2.344186	287.54***
Months (M)	2	.172950	21.21***
V × S	4	.114859	14.09***
V × M	4	.010657	1.31
S × M	4	.0017362	(NS)
V × S × M	8	.0081525	
Total	26		
Varieties			
(a) Swedes vs. Chou moellier and Rape	1	2.999094	367.87***
(b) Chou moellier vs. Rape	1	.071442	8.76**
Varieties × Sites			
(a) vs. Sites	2	.177846	21.81***
(b) vs. Sites	2	.051872	6.36**
Sites			
Annat vs. Lincoln and Darfield	1	4.686968	374.91***
Lincoln vs. Darfield	1	.001405	(NS)

TABLE OF MEANS

	Lincoln	Darfield	Annat	Overall mean
Swede	.244	.321	.832	.466
Chou moellier	.954	.923	1.831	1.236
Rape	.757	.657	1.916	1.110
Overall mean	.652	.634	1.527	.937

TABLE 11. ANALYSIS OF VARIANCE OF INTEGRATED MONTHLY MEAN PLANT WEIGHTS FOR ALL SITES AND HOST VARIETIES (per plant)

Source of variation	df	MS	F
Varieties (V)	2	21387.85	44.61***
Sites (S)	2	30519.35	63.65***
Months (M)	11	12030.01	25.09***
V × S	4	8283.75	17.28***
V × M	22	1344.33	2.80**
S × M	22	2450.80	5.11***
V × S × M	44	479.46	
Total	107		
Sites			
Lincoln vs. Darfield and Annat	1	50477.8	105.03***
Darfield vs. Annat	1	10560.9	22.02***

TABLE OF MEANS

	Lincoln	Darfield	Annat	Overall mean
Swede	56	119	81	85
Chou moellier	58	159	99	105
Rape	44	52	77	57
Overall mean	52	110	86	83

Although the sites differed considerably in detail, nevertheless an overall pattern of aphid population fluctuation was apparent. There were two major peaks of aphid numbers: the first in late summer or autumn corresponding to the maximum of vegetative growth of the hosts, and a second larger peak the following spring corresponding to flowering and seeding of the biennial host plants. Aphid numbers were low in winter when temperatures were low, bulb formation had taken place, and the tops of the hosts had declined.

The abundance of alate forms in traps or on the host plants paralleled the occurrence of peak aphid populations on the plants. Thus there were two major peaks as above.

The growth of aphid populations on the different plant varieties at the different sites will now be considered in terms of this overall pattern. In order to emphasise the pattern of population change and to smooth out minor fluctuations, the mean log (n + 1) apterae per five plants was calculated for each variety for each month by graphical integration. These monthly means are illustrated in Figs. 5, 6, and 7. Analysis of variance of these means (Table 9) revealed highly significant differences between host

varieties, between sites, and between months, as well as highly significant interactions: varieties × sites and sites × months.

Considering the year's results as a whole, greatest aphid numbers were found at Annat, while lowest aphid numbers were found on swede plants irrespective of site. When the monthly

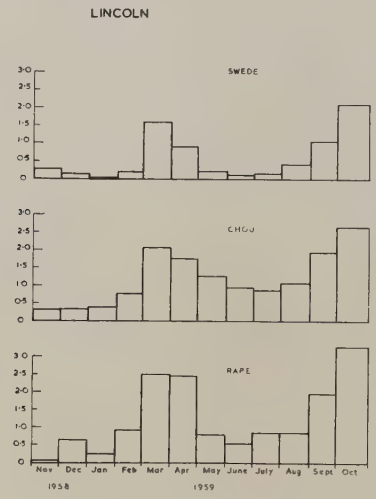

Fig. 5.—*Lincoln:* integrated monthly mean log (n + 1) apterous *B. brassicae*/5 plants on swede, chou moellier, and rape.

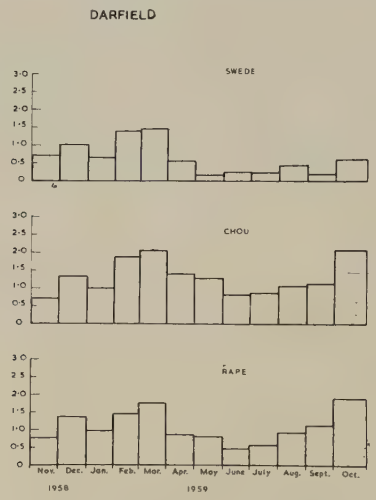

Fig. 6.—*Darfield:* integrated monthly mean log (n + 1) apterous *B. brassicae*/5 plants on swede, chou moellier, and rape.

means are examined (Figs. 5, 6, and 7) some interesting variations appear. A notable difference between sites lay in the different levels of winter aphid populations.

Analysis of variance of the integrated monthly means (as log (n + 1) apterae per five plants) for the months of June-August is summarised in Table 10.

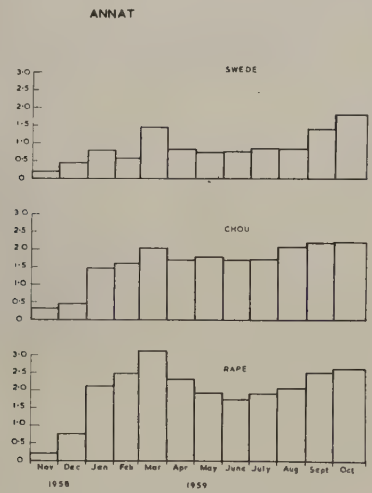

Fig. 7.—*Annat:* integrated monthly mean log (n + 1) apterous *B. brassicae*/5 plants on swede, chou moellier, and rape.

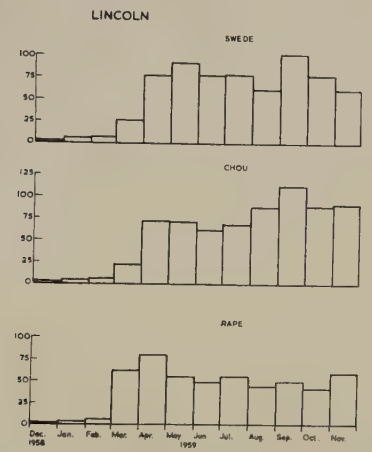

Fig. 8.—*Lincoln:* integrated monthly mean plant weights of swede, chou moellier, and rape (g per plant).

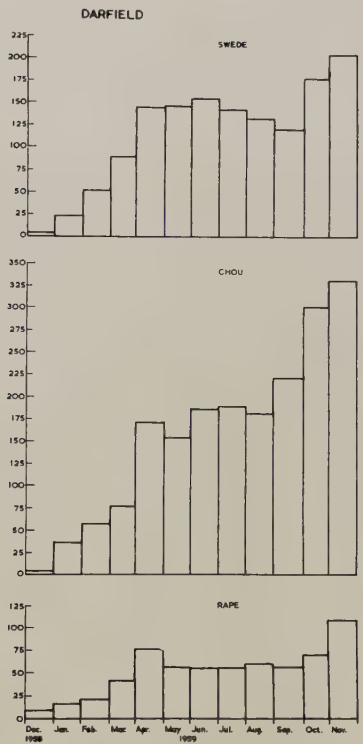

Fig. 9.—*Darfield:* integrated monthly mean plant weights of swede, chou moellier, and rape (g per plant).

Highest winter populations were encountered at Annat, while the differences between Lincoln and Darfield were not significent. In winter aphids were fewest by far on swedes at all sites, and at Lincoln and Darfield more were found on chou moellier than on rape.

Growth of the host plants was measured by plant weight determined at each sampling. The mean weights are summarised in Tables 6–8. In addition, the integrated monthly means are illustrated in Figs. 8, 9, and 10. The results of analysis of variance of the integrated monthly mean plant weights are summarised in Table 11.

Differences between varieties, sites, and months were highly significant, as were the first order interactions between these variables. Rape was the lowest-yielding variety at each site, the difference between this and the other varieties being most marked at Darfield. Highest yields of swede and chou moellier were obtained at Darfield, followed by Annat.

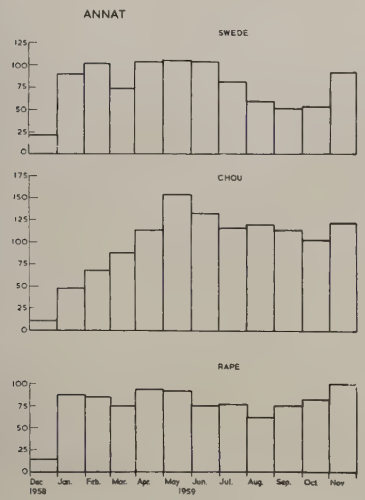

Fig. 10.—*Annat:* integrated monthly mean plant weights of swede, chou moellier, and rape (g per plant).

DISCUSSION AND CONCLUSION

In most cases the second (spring) population peak was greater. At this time brassica field crops produce new shoots which bear flowers and seed. The flower stalks are preferred sites for aphid colonisation (especially towards the tips where the flowers mature first). This preference is most probably associated with intense translocation of nutrients into the developing seeds. As seed formation progresses dense aphid colonies develop. As the seed ripens an increasing proportion of aphid progeny becomes alate and ultimately flies from the host plant. This results in mass spring migration of aphids, which colonise newly sown brassica crops—and incidentally carry over viruses from diseased plants which have survived to the flowering stage. Viruses can also be picked up and transmitted in the course of these migratory flights. Thus spring migration resulting from overcrowding and competition for nutrients in the seeding plants ensures the survival of the species by infestation of new young plants.

This migration was responsible for the small peak in aphid numbers observed early in the season at Darfield and Lincoln. Immigration was greatest at Darfield where the trial was located close to a flowering chou moellier crop. It was less at Lincoln where fewer sources of infestation were nearby, and was least apparent at Annat where (as shown in Fig. 4) flying aphids were less abundant than at the other sites in October and November 1958. Detailed results of alate aphid trapping at these sites have been presented by Close and Lamb (1961).

Thus it would seem that the intensity of initial aphid infestation is largely determined by proximity to sources of flying aphids but, as the above results demonstrate, the ultimate intensity of aphid infestation is not necessarily determined by this factor since the greatest aphid numbers were found at Annat where initial infestation was light.

The effects of aphid infestation on the hosts, and *vice versa*, will be considered later. Also the effect of other environmental factors on aphid population growth and various correlations will be considered in detail in a later Part of this series. Detailed interpretation of results would therefore be premature at this stage.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of officers of Applied Mathematics Laboratory, D.S.I.R., in statistical treatment of data. Thanks are also due to Messrs F. Jarman of Darfield and G. Wright of Annat, on whose properties trial plots were located.

REFERENCES

Close, R. 1959: Sticky traps for winged aphids. *N.Z. J. agric. Res. 2:* 375–9.

Close, R.; Lamb, K. P. 1961: Trapping study of some winged aphid vectors of plant virus diseases in Canterbury, N.Z. *Ibid. 4:* 606–18.

Garnier, B. J. 1958: "The climate of New Zealand." 1st ed. pp 51–2. E. Arnold, London. 191 pp.

Hayman, B. I.; Lowe, A. D. 1961: The transformation of counts of the cabbage aphid (*Brevicoryne brassicae* (L.)). *N.Z. J. Sci. 4:* 271–8.

Jacob, F. H. 1958: Some modern problems in pest control. *Sci. Progr. Twent. Cent. No. 181:* 30–45.

Lamb, K. P. 1960: Field trial of eight varieties of brassica field crops in the Auckland district. I. Susceptibility to aphids and virus diseases. *N.Z. J. agric. Res. 3:* 320–31.

Lowe, A. D. 1958: Insect pests of brassicas. *Proc. 8th Lincoln Coll. Frmrs' Conf.* pp. 111–20.

Milne, A. 1957: Theories of natural control of insect populations. *Cold Spring Harbour Symposia on Quant. Biol. 22:* 253–71.

Morris, R. F. 1960: Sampling insect populations. *Ann. Rev. Ent. 5:* 243–64.

THE OVER-WINTERING OF THE CABBAGE APHID (*Brevicoryne brassicae*) ON FARM CROPS IN THE SOUTH ISLAND OF NEW ZEALAND

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(Received 11 July 1961)

ABSTRACT

As part of a long-term study of the cabbage aphid as it affects farm crops in the South Island, a survey was made of the farm crops of brassicas still standing in July and August 1958, covering Ashburton County, and in July and August 1959, covering the Rakaia-Waimakariri area. A total of 237 paddocks was sampled, and the data subjected to analysis of variance. Populations on turnips were significantly lower at the 1 per cent level than on other crops, which showed no significant differences. Weeds played little part in over-wintering of the pest, and rape seed crops were shown to receive their initial infestation from small numbers of alates flying in winter.

Utilisation of crops during winter leaves chou moellier, rape, and weeds as the sources of spring infestation. The implications of these facts for control are briefly discussed.

INTRODUCTION

Brassicas are an important part of farm feed crops in New Zealand, about 750,000 acres being grown annually. Of this total 70 per cent are grown as supplementary feed in the South Island, a total of 300,000 acres being grown in Canterbury.

The problem of the cabbage aphid in these crops is periodically a serious one.

In addition to direct feeding by the aphid, damage is also done by the virus diseases popularly known as "turnip mosaic." Diseases included in this term are cabbage black ring spot (the "turnip mosaic" mentioned by Chamberlain (1954)) and cauliflower mosaic. The cabbage aphid is an acknowledged vector of both these diseases (Broadbent 1957).

The problem is common to the South Island and parts of the North Island of New Zealand, and is to some extent accentuated by the dry climate throughout Canterbury. It is in this area also that rape for lamb fattening and turnips for winter feed on the light loamy soils are important feed crops at critical periods of the year. In some years the turnip crops have been virtually wiped out by cauliflower mosaic, the more destructive of the two diseases.

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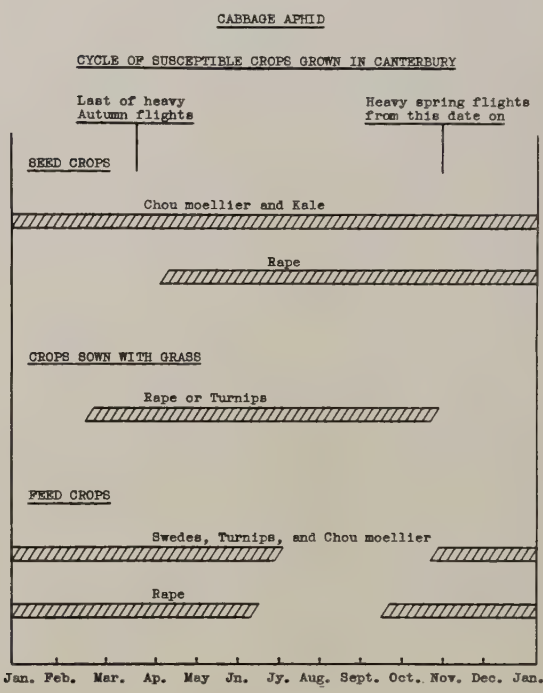
CABBAGE APHID
CYCLE OF SUSCEPTIBLE CROPS GROWN IN CANTERBURY
Last of heavy Autumn flights
Heavy spring flights from this date on
SEED CROPS
Chou moellier and Kale
Rape
CROPS SOWN WITH GRASS
Rape or Turnips
FEED CROPS
Swedes, Turnips, and Chou moellier
Rape
Jan. Feb. Mar. Ap. May Jn. Jy. Aug. Sept. Oct. Nov. Dec. Jan.

Fig. 1.—The succession of brassica crops throughout the year in Canterbury.

Growers contribute to the problem by providing a succession of brassica crops throughout the year (see Fig. 1):

(a) Seed crops, particularly of chou moellier, are present in the ground for a full year, culminating in the harvesting of seed in December, and could be playing an important part in over-wintering of the pest.

(b) Crops such as swedes, turnips, and chou moellier grown for feed are normally fed off by mid-September at the latest, but occasionally the last may be retained for seed, and at times the others may be left in the ground to seed and could become a source of infestation.

(c) Rape and turnips are sometimes sown lightly with autumn-sown grass. Such grass is frequently not heavily grazed until it has made plenty of spring growth, and in many cases the residue of brassicas could assist in the over-wintering of the pest.

(d) Other sources of infestation could be cruciferous weeds, and residues of crops such as savoy cabbage, brussels sprouts, and cauliflowers in farm or market gardens.

EXPERIMENTAL METHODS

In order to gain accurate data on the part played by farm crops in the over-wintering of this pest surveys were undertaken in two important brassica-growing areas in Canterbury in July and August of both 1958 and 1959. No attempt was made to survey market garden crops because this problem, though possibly contributory, was felt to require separate assessment. The areas chosen were: 1958 -Ashburton County; and 1959—the area lying between Rakaia and Waimakatiri rivers. The areas are shown in Figs. 2 and 3, and their localities have been indicated in figures by Lamb and Lowe (1961). These figures show elevation contours at 400, 800, 1,200, and 1,500 ft above sea level, together with the actual locations of paddocks sampled. Reference to the locality map of the South Island (Lamb and Lowe *loc. cit.*) will show that normally Banks Peninsula falls within the area shown in Fig. 3. However, in this survey Banks Peninsula was excluded, since it represents an entirely different ecological unit with a different type of farming.

As far as possible the aim was to give even coverage to each area. This was, however, sometimes limited by the number of crops still standing in July and August in particular areas.

Crops

Total cabbage aphids per plant were counted on 10 samples drawn at 20 pace intervals on a random transect in each paddock. Only occasional alates were present, and the totals consist mainly of a count of apterous adults and associated nymphs. A total of 237 paddocks was sampled, and the transformed data were subjected to analysis of variance. A summary of the results is set forth in Table 1.

TABLE 1. 1958–9 WINTERS—SAMPLING OF THE CABBAGE APHID IN FIELD CROPS

Variety	No. of paddocks sampled	Mean log (n + 1)	Equiv. aphids per plant	Significant differences by Duncan's test*	
				5%	1%
Turnips	91	.17	0.5	a	A
Rape	44	.92	7.3	b	B
Swedes	36	.99	8.9	b	B
Chou moellier	66	1.37	22.5	b	B
		s.d. = .148			

* Means designated by the same letter are not significantly different.

Weeds

The cabbage aphid confines its attentions mainly to the genus *Brassica,* but includes a few other plants of the Cruciferae within its

host range. Weeds within this range of plants were therefore examined, and sampled on a basis similar to that used for crop plants. Results of this part of the survey are summarised in the text.

Rape seed crops

Reference to Fig. 1 indicates that rape seed crops are planted in April, subsequent to autumn flights of the cabbage aphid. This planting technique should avoid infestation until the following spring. In practice, however, these crops are frequently found to be heavily infested in patches with apterae and in need of spray treatment as early as September, i.e., before heavy spring flights occur (Lamb and Lowe *loc. cit.*). Four such crops were available for sampling in the Ashburton area in 1958, and these were sampled by a more extensive system of 40 samples per paddock. Results of this sampling are summarised and their significance discussed later.

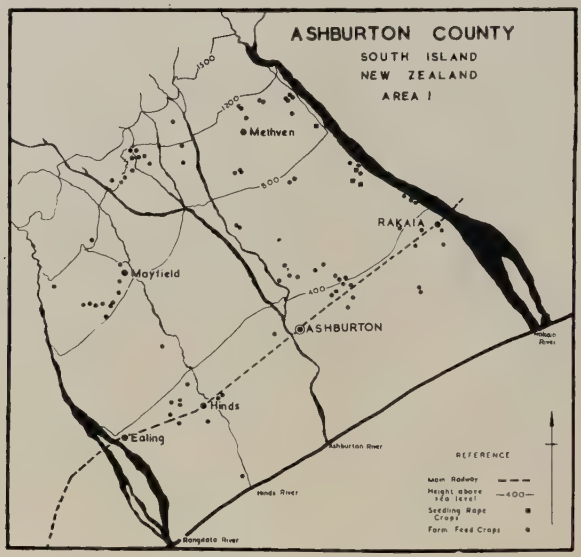

Fig. 2.—Ashburton County: sampled 1958.

RESULTS

Crops sown with grass or lucerne, or in combination with other brassica varieties, showed no consistent differences in infestation from those sown as pure stands. Analysis of variance failed to reveal any significant differences between areas or altitudes. The data were therefore examined as a whole for differences between varieties. This examination revealed that turnips carried low winter populations which differed significantly, at the 1 per cent level, from all other varieties. The differences between chou moellier and rape fell just short of significance at the 5 per cent level.

Weeds

A separate preliminary survey, to establish the general incidence of cruciferous weeds in the Ashburton area, is shown in Table 2. Each paddock was transect-sampled at 10 pace intervals. Weeds not listed occurred in fewer than 1 per cent of the areas sampled, including all cruciferous weeds. This finding is in agreement with the fact that in the 237 paddocks sampled such weeds were present in sufficient numbers for sampling (10 plants) on only 41 occasions. In addition, 12 other paddocks carried occasional plants of shepherd's purse. All available plants were sampled, and in occasional weedy paddocks up to 100 plants were examined. Only in one case, that of wild turnip carrying a flower-head, was the cabbage aphid found on a weed.

In this case a single alate was found in the flower-head.

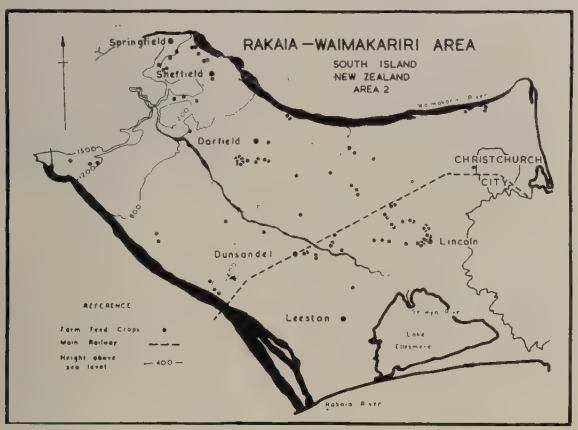

Fig. 3.—Rakaia–Waimakariri area: sampled 1959.

Of the 41 paddocks carrying weeds sufficient for routine sampling, 31 sets of samples were from shepherd's purse (*Capsella bursa-pastoris* (L) Medik.), 7 were from wild turnip (*Brassica campestris* L.), and 3 from hedge mustard (*Sisymbrium officinale* (Linn.) Scop.). It is of interest to note that the first of these weeds was the only one to appear in the 1958 sampling in Ashburton County.

Though wild turnip is not a prolific weed in urban areas, numbers of plants may be found in waste places in and near townships. A separate sampling of such areas near Christchurch city showed that of 400 plants sampled in 20 different sites, some 80 per cent of stems carried considerable populations of the cabbage aphid approaching and during flowering and seeding. No cabbage aphids were found on other cruciferous weeds in these areas, though such weeds were not as plentiful as wild turnip in the late winter.

TABLE 2. ASHBURTON COUNTY—INCIDENCE OF WEEDS IN 200 PADDOCKS

Over 5% incidence		1–5% incidence	
Species	Percentage incidence	Species	Percentage incidence
Hypochaeris radicata Linn.	13	*Senecio jabobaea* Linn.	4
Verbascum thapsus Linn.	11	*Cirsium arvense* (L.) Scop.	2
Cerastium caespitosum Gilib.	10	*Rumex obtusifolus* Linn.	1
Plantago lanceolata Linn.	8	*Spergula arvensis* Linn.	1
Rumex acetosella Linn.	7		

Rape seed crops

Four rape seed crops were available for sampling. All were in the rosette stage, having been sown late in April. In no case did the usual 10 samples reveal any aphids. However, when this process was repeated 4 times the 40 samples twice revealed small numbers of aphids. In one paddock 2 alates were taken. In a second paddock 2 alates, one of which was associated with 2 apterae, were found. The remaining 2 paddocks still gave nil returns.

DISCUSSION

It is clear from the results presented that turnips are not a favoured over-wintering site for the cabbage aphid in Canterbury. Of the other crop plants sampled, chou moellier is the most heavily colonised; and differences, though consistent, just fail to reach significance. It should be noted that chou moellier is a much larger plant than either of the varieties with which it is here being compared.

The part played by such crops in carrying over-wintering populations forward to the spring period is intimately associated with the farm utilisation of such crops. As indicated earlier, such crops may be for feed, or sown with grass, or purely for seed production. The first two types of crop are normally fed off as winter feed for sheep in July or August. Under these circumstances, they would play no part in the spring flights. If left to run up to seed, either through carelessnes or intention, they can then be classed as seed crops, and play an important part in carrying forward an initial spring population, as do those crops intentionally planted for seed production. Under the seed certification scheme conducted by the Department of Agriculture in New Zealand, chou moellier, rape, and a very limited acreage of kale are the only brassica crops deliberately grown for seed in the area sampled.

The incidence of weeds suitable as host plants for this aphid during winter is very low, and this limits the population available from this source.

The infestation of rape seed crops planted in late April has offered some puzzling aspects, especially if the flights of this aphid are regarded as ceasing early in April. Such considerations, however, are based on gross flight data taken from sticky traps (Lamb and Lowe *loc. cit.*). The same authors have shown that alates may be present on plants in small numbers during winter, though not taken on traps. Thus the taking of small numbers of alates on seedling rape crops in winter, by more extensive sampling, is not surprising. Maximum daily temperatures in open sites in Canterbury during July and August 1959 exceeded 58°F on six occasions, and 60°F on two occasions. As aphid take-off has been observed by the author at temperatures as low as 60°F in the field several times at very low wind-speeds, it seems feasible that alates would fly in small numbers during calm winter days. Temperatures such as those recorded above are also adequate for growth and reproduction in the cabbage aphid, and this would permit the early build-up of apterae following small flights of alates.

CONCLUSIONS

General. It is clear from the data and discussion above that chou moellier seed crops are a major source of infestation of the cabbage aphid in spring brassica crops in Canterbury. Turnips are unimportant, while crops of rape and swedes that are not utilised by the end of August could also fill an important role in carrying forward an initial population.

Except for wild turnip, which is not a weed of high incidence in many rural areas in Canterbury, no significant part is played by weeds in the over-wintering of this pest in Canterbury.

While the limited figures for rape seed crops are not to be regarded as a survey, such crops do appear to become infested by small numbers of alates during the winter, and it is from these that the early spring build-up in this crop could originate. Such initial populations could become an additional source of alates by the normal flight period beginning about mid-September.

Control. These facts suggest that a significant reduction in spring flights would be likely to occur if all seed crops were sprayed with a reliable aphicide before the beginning of September. Such measures would need to be supplemented by the destruction of other crops not required and of wild turnip.

The period of the year under survey is the time at which the populations of this pest reach their lowest level. This fact might well be considered in any programme for the biological control of this pest.

ACKNOWLEDGMENTS

The author wishes to thank officers of the Applied Mathematics Laboratory, D.S.I.R., Lincoln, for analysing the data, and Mr A. J. Healy, of the Botany Division, D.S.I.R., Lincoln, for the identification of weeds.

REFERENCES

Broadbent, L. 1957: "Virus diseases of brassica crops." pp. 36–7. C.U.P. 94 pp.

Chamberlain, E. E. 1954: Plant virus diseases in New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 108:* 153–63.

Lamb, K. P.; Lowe, A. D. 1961: Studies of the ecology of the cabbage aphid (*Brevicoryne brassicae* (L.)) on brassica field crops in Canterbury, New Zealand. I. Introduction and population study, 1958–9. *N.Z. J. agric. Res. 4:* 619–42.

WINTER GROWTH OF LUCERNE (*Medicago sativa*)

III. Variety production at Lincoln, New Zealand

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(Received 23 June 1961)

ABSTRACT

A range of lucerne varieties, predominantly from areas with Mediterranean climates, was grown in trials at Lincoln. Many varieties yielded more dry herbage than New Zealand Certified in the late autumn (April–May), and some Spanish and a few other varieties yielded more in late winter (June–August).

These winter-active varieties could be valuable to farmers growing lucerne on soils where summer production of lucerne is limited by drought.

INTRODUCTION

In recent years lucerne culture has been considerably extended, particularly on to areas of shallow soils which overlie deep gravel beds. These areas, known locally as "light land," suffer various degrees of drought in late spring, summer, and autumn.

Rickard (1960) has calculated periods of soil moisture deficits on the Lismore soil type, which in its moisture relations is a typical "light land" soil, with the 30 cm of top-soil holding 5 cm of available water at field capacity. Though lucerne roots penetrate the matrix of sand and gravels which underlies these soils, they evidently do not obtain sufficient moisture for active growth, and the results published by Rickard suggest that on these soils summer drought limits the production of lucerne as much as other pasture species.

In many seasons growth is limited by drought on these soils for much of the period from October to April. From May to September, however, moisture is usually adequate, and for intensive farming it is necessary to make full use of this water. This is achieved by using pastures of winter-growing ryegrass and subterranean clover, and by lambing early in the season, so that lambs may be sold before the onset of drought, and feed requirements are at a minimum level from January to June. The late winter, with pregnant ewes to be fed, is a critical period.

In this environment there is great variation in pasture production from year to year. Rickard (*loc. cit.*) recorded yearly lucerne produc-

tion varying from 4,060 lb of dry matter per acre to almost 10,000 lb, while Iversen and Calder (1956) reported similar variations in pasture production.

Lucerne on light land is used for grazing during periods of pasture shortage in late winter and summer, and provides hay for feeding in the winter, and in years of low production.

Palmer (1959) has shown that lucerne varieties differ considerably in their elongation rates over the winter period. In this paper, yields of herbage obtained from April to the end of August from variety trials at Lincoln are reported.

EXPERIMENTAL

Material

Individual lines, varieties, or ecotypes are listed in the tables. Most of the material was obtained from Mediterranean countries, or from other areas with Mediterranean climates.

All trials included New Zealand Certified (Strain B) lucerne, which was bred in New Zealand, and is known to be a persistent productive strain; Du Puits, which has about the same growth period as New Zealand Certified (Palmer *loc. cit.*); and Provence, which grows more actively in the winter. Two trials contained Rhizoma from Canada, and a strain of *Medicago glutinosa* from Canterbury Agricultural College, Lincoln; these have both been extensively recommended for use on light land.

Marlborough, a local variety, may have developed as an ecotype in New Zealand (Hadfield and Calder 1936). Maniototo is another local strain which has been grown for at least two crop generations in a region with colder winters than the main lucerne growing areas.

Some of the seed samples were obtained from research stations, while others were samples of commercial seed imported into New Zealand. These included a number of unnamed importations from France, which have been labelled as putative Provence or Flamande lucernes. Lucernes without variety names are listed by place or origin.

Soil type

The soil on which the trials were sited is classified as Templeton Stony Silt Loam. A profile from a nearby pit is shown in Fig. 1.

The depth of top soil over the trial area varied considerably, causing marked variability in summer production, but not greatly affecting winter production.

The trials

Trial 1 included 25 entries. Plots were of 6 rows 15 cm apart and 15 metres long, with 30 cm between plots. The trial consisted of four randomised blocks, and was sown on 29 September 1955.

Fig. 1.—Profile of soil similar to that under trial area. (Scale in inches).

Trials 2 and 3 each contained 36 entries arranged as a 6 × 6 lattice with four replications. Results from one unnamed variety in both trials have been omitted. Plots consisted of five rows five metres long, with 18 cm between rows, and 36 cm between plots. Trial 2 was sown on 31 October 1956, and Trial 3 on 27 March 1957.

All plots were sown at 11 kg of viable seed per hectare (10 lb/acre), and were top-dressed annually in March with about 125 kg of superphosphate per hectare (100 lb/acre).

Growth periods

Plots were cut in late March each year, and production from each was determined in late May. Production was again measured in late August or early September. Autumn production figures are available for 1957, 1959, and 1960, and late winter production for 1959 and 1960. In 1959 plots in Trial 2 were split, half of each was harvested on 20 August, and the other half on 7 September.

Harvesting

Up to the harvest of May 1959, plots were cut as close as possible to ground level with a sickle-bar mower. From the spring of 1959, plots were cut with a roto-scythe to within about 3 cm of ground level. Produce from the whole plot was weighed, and a 500 g sample dried at 80°c for dry matter determinations. All yields are of the total herbage from the plot, which includes some annual weeds, and a small amount of volunteer rye-grass (*Lolium* spp.), cocksfoot (*Dactylis glomerata* L.), and brown-top (*Agrostis tenuis* L.).

The seasons

Rainfall, mean monthly temperatures, and hours of bright sunshine at Canterbury Agricultural College, Lincoln, for the relevant periods are given in Table 1.

TABLE 1. MEAN TEMPERATURES, HOURS OF BRIGHT SUNSHINE, AND RAINFALL FROM MARCH TO AUGUST AT LINCOLN

	Year	Month					
		March	April	May	June	July	August
Mean temperature (°C)	1957	15.8	12.4	8.8	6.1	5.2	7.3
	1959	14.8	12.2	6.0	5.5	5.1	6.1
	1960	13.6	11.9	8.8	6.8	6.3	6.2
	Station average	13.9	11.4	8.1	5.8	5.0	6.3
Hours bright sunshine	1957	188	137	139	126	137	144
	1959	172	177	109	152	157	191
	1960	156	183	126	105	146	151
	Station average	179	143	132	112	115	143
Rainfall (mm)	1957	119	100	160	33	95	79
	1959	140	72	197	10	32	7
	1960	128	34	13	145	33	32
	Station average	55	49	63	64	69	50

Temperatures were slightly above average in 1957. In 1959, April was slightly warmer than average, and May was 2.1°c below average. April and May of 1960 were somewhat warmer than average. June, July, and August 1959, and April 1960 had much more bright sunshine than usual. There was sufficient moisture for vigorous growth in each year.

RESULTS

Results from Trials 1, 2, and 3 are presented in Tables 2, 3, and 4 respectively. Yields from each harvest were analysed separately, and significant differences calculated using the multiple range test proposed by Duncan (1955). To condense the tables, yields of varieties of similar origin and performance have been averaged. The differences required for significance in comparing individual varieties by the *t*-test are given in the tables.

Late autumn production

1957. (Table 2). One line of Provence, one of Du Puits, and Caliverde produced significantly more than New Zealand Certified. The three lines of Hunter River and the five from Alessandria, Italy (which were probably identical) and Caliverde produced more than New Zealand Certified. Rhizoma produced significantly less than New Zealand Certified.

TABLE 2. YIELDS OF DRY MATTER IN KG/HECTARE FROM LUCERNE VARIETIES IN TRIAL 1

Variety	Entries	Yields				
		25/5/57	20/5/59	26/5/60	2/9/59	23/8/60
New Zealand Certified	1	970	980	784	1,940	1,021
Marlborough	1	970	1,147	999*	1,955	1,085
Maniototo	1	988	1,082	889*	2,069	1,119
Provence	5	876	1,076	1,142*	1,924	934
Provence	1	1,221*	1,572*	1,643*	2,034	781*
Flamande	3	1,005	1,048	949*	1,998	898
Du Puits	1	1,221*	999	896*	1,865	795*
Du Puits	1	793	906	784	1,594	605*
Hunter River	3	1,181	1,559*	1,645*	1,857	870
Alessandria, Italy	5	1,186	1,505*	1,518*	1,900	795*
Caliverde	1	1,259*	1,590*	1,699*	1,742	743*
Rhizoma	2	690*	555*	373*	1,002*	687*
Significant difference between adjacent entries (5% level)		242	215	94	520	162

* Significantly different from New Zealand Certified

1959. (Tables 2, 3, and 4). Comparisons are available both within and between trials. The lines from Alessandria yielded significantly more than New Zealand Certified in all trials. South African and Saladina were considerably better in both trials in which they were included. Orange, Hunter River, Provence (1 line), California Common, New Mexico Common, Caliverde, and Bolivia yielded more than New Zealand Certified. Du Puits gave a yield similar to that of New Zealand Certified in three trials, while *M. glutinosa* yielded more in one trial, less in another.

1960. (Tables 2, 3, and 4). In trial 1 Rhizoma yielded less than New Zealand Certified, one line of Du Puits the same, and all others more. In Trial 2 *M. glutinosa* yielded less, and all except Chartrainvilliers significantly more. In Trial 3 Provence, South Africa, Indian, Caliverde, Hunter River, Orange, Friulana, and Alessandria produced significantly more, *M. glutinosa* significantly less.

The differences between the high-yielding varieties and New Zealand Certified were greater than they were in 1957 and 1959, and some varieties which produced about the same as New Zealand Certified in 1959 produced considerably more in 1960. These included seven ecotypes of Spanish lucerne, African, New Mexico 11–1, the two lines of Du Puits, and the two of Provence from Trial 2, two of Provence, and Friulana from Trial 3.

Late winter production

Many varieties yielded more than New Zealand Certified in April and May, but only a few did from June-August.

In Trial 1 no varieties produced significantly more in either year, and Rhizoma produced less in both. In Trial 2, African and the seven Spanish ecotypes produced more on 20 August 1959, and on 7 September the Spanish ecotypes and New Mexico 11-1 produced more, while *M. glutinosa* and Bolivia produced less. On 22 August 1960 one line of New Mexico Common and New Mexico 11-1, African, and the Spanish lucernes produced more, *M. glutinosa* and Bolivia less. In Trial 3 no varieties produced more than New Zealand Certified, and Oklahoma, Indian, Orange, Saladina, Cremonese, Rhizoma, Grimm, and *M. glutinosa* less.

TABLE 3. YIELDS OF DRY MATTER IN KG/HECTARE FROM LUCERNE VARIETIES IN TRIAL 2

Variety		En-tries	Yields				
			21/5/59	25/5/60	20/8/59	7/9/59	22/8/60
New Zealand Certified		3	1,313	1,011	889	2,221	751
Provence		2	1,255	1,459*	1,014	2,217	873
Du Puits		2	1,322	1,362*	820	2,142	647
Chartrainvilliers		1	1,255	1,193	852	2,203	855
South Africa		10	1,666*	1,750*	1,018	2,172	674
Alessandria, Italy		1	1,862*	2,188*	1,079	2,118	659
Saladina, Argentine		1	1,942*	1,756*	1,066	2,157	763
California Common	U.S.A.	1	1,667*	1,958*	1,043	2,253	670
Caliverde		1	1,524	1,569*	1,072	2,192	682
New Mexico 11-1		1	1,336	1,409*	1,083	2,400*	879*
New Mexico Common		1	1,613*	1,850*	1,118	2,372	948*
Indian		1	1,522	1,778*	1,079	2,121	682
African		1	1,381	1,621*	1,173*	2,280	879*
Spain		7	1,220	1,983*	1,235*	2,570*	1,155*
Bolivia		1	1,680*	1,380*	953	1,907*	509*
Medicago glutinosa		1	1,208	575*	566*	2,035*	705
Significant difference between adjacent entries (5% level)			294	333	240	171	120

* Significantly different from New Zealand Certified

DISCUSSION

Late autumn production

The results have shown that there are many varieties which have consistently yielded considerably more than New Zealand Certified in April and May. Mediterranean lucernes, or lucernes from areas with Mediterranean climates, figure prominently in this group, which also

TABLE 4. YIELDS OF DRY MATTER IN KG/HECTARE FROM LUCERNE VARIETIES IN TRIAL 3

Variety		Entries	21/5/59	26/5/60	27/8/59	24/8/60
New Zealand Certified		2	864	976	1,233	969
Chartrainvilliers		1	854	999	1,336	877
Du Puits		1	916	1,053	1,059	813
Flamande		7	923	1,293	1,309	1,050
Provence		2	860	1,498*	1,380	1,058
South Africa		1	1,251*	1,920*	1,285	897
Oklahoma	U.S.A.	1	773	858	940*	851
Williamsburg	U.S.A.	1	916	922	1,113	911
Buffalo	U.S.A.	1	784	759	1,001	910
Indian	U.S.A.	1	1,109	1,714*	1,224	717*
African	U.S.A.	1	906	976	1,283	906
Caliverde	U.S.A.	2	1,119	1,594*	1,245	892
Hunter River	Australia	1	997	1,541*	1,334	1,115
Orange	Australia	1	1,393*	1,855*	1,117	728*
Saladina, Argentine		1	1,303*	1,356	1,061	687*
Romagnola	Italy	1	926	1,161	1,168	903
Cremonese	Italy	1	392*	1,031	959	706*
St. 202	Italy	1	673	1,019	1,026	806
Leonicena	Italy	1	813	1,096	1,055	814
Friulana	Italy	1	874	1,389*	1,184	951
Pastore	Italy	1	997	1,280	1,276	992
Alessandria	Italy	1	1,434*	1,964*	1,292	785
Rhizoma		2	620	641	558	476*
Grimm		1	692	716	903*	800
Medicago glutinosa		1	1,078	521*	1,018	683*
Significant difference between adjacent entries (5% level)			314	392	277	197

* Significantly different from New Zealand Certified

includes some lucernes adapted to more tropical conditions, such as Saladina, Indian, and African.

The Spanish lucernes produced about the same as New Zealand Certified in 1959, and about twice as much in 1960. Other more productive varieties produced relatively much more in 1960 than in 1959. The difference in behaviour in the two years may be caused by variation in seasonal conditions, but has not been related to variation in any one factor.

Late winter production

While autumn production can be assessed when all varieties have ceased growing actively, the date of harvest may appreciably affect the assessment of late winter production from different varieties. If harvest is delayed until late September or early October, varietal differences may be small, or non-existent (Palmer unpublished data). In late July and early August, when some varieties have started growing while others have not, differences will be considerable. Until the seasonal production curves of different varieties in the late winter are known, there must remain some doubts about variety assessments made from cutting at one date. The results from Trial 2, which was harvested on 20 August and 7 September 1959, show that the relative order of varieties may be different at different harvest dates in the same season (Table 3).

However, it would seem reasonable to measure production during that period of the late winter when feed production is still below requirements, as then relatively small differences in production will be of economic significance, while later in the season, when there is a surplus of feed, differences will be of less economic importance.

Time of harvest is then a matter of judgement, and the harvest dates used here in late August and early September are at the end of the winter shortage period, and the beginning of the spring surplus. In fact, assessment at earlier dates might yield data of more economic significance.

Only a few varieties have yielded more in the late winter than New Zealand Certified, and many which yielded more in the late autumn did not yield more in the late winter. This contrasts with variations in stem elongation of different varieties (Palmer 1959), which were highly correlated in late autumn and late winter. This lack of symmetry in relative production in late autumn and late winter must be attributed to differences in varietal reactions to environmental variations.

Total winter production

These trials have shown that some varieties are capable of higher production from April to August than varieties which have been bred here such as New Zealand Certified, or than ecotypes which may have evolved here, such as Maniototo or Marlborough. Whether the more winter-active lucernes have a shorter life in this area is not known. There is no evidence of this from these trials, which have only been

run over a short period, and exceptional seasons may have a large selective influence.

Rhizoma, Grimm, and *M. glutinosa* produced relatively little over the winter period, and these lucernes are more winter dormant than necessary for this area.

Those varieties which grew most actively as judged by increases in stem length did not necessarily produce the most. The differences in numbers of stems per unit area which were recorded for spaced plants (Palmer *loc. cit.*) were apparent also in these swards, and none of the more active varieties also produced a high number of stems per unit area.

CONCLUSIONS

Some lucernes from the Mediterranean, or from areas with Mediterranean climates, or from low latitudes such as New Mexico or Australia, produced more in the late autumn than New Zealand Certified, and some produced considerably more in one year, but not in another.

From late May to the end of August or early September most of those lucernes did not produce appreciably more than New Zealand Certified, but a range of Spanish ecotypes did so. These gave increments in winter production which would be valuable to New Zealand farmers growing lucerne on light land where growth over the summer is limited by drought.

Strongly winter dormant varieties such as Rhizoma and Grimms yielded much less than New Zealand Certified over the winter period.

Because of variability from season to season it is suggested that trials over a larger number of years are necessary for a complete assessment of the relative performance of these lucerne varieties.

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REFERENCES

Duncan, D. B. 1955: Multiple range and multiple *F.* tests. *Biometrics 11:* 1–43.

Hadfield, J. W.; Calder, R. A. 1936: Breeding and selection of lucerne (*Medicago sativa*) *N.Z. J. Sci. Tech. 17:* 659–65.

Iversen, C. E.; Calder, J. W. 1956: Light land pastures. *Proc. 18th Conf. N.Z. Grassl. Ass.* pp. 78–88.

Palmer, T. P. 1959: Winter growth of lucerne (*Medicago sativa*). I. Variety performance in New Zealand. *N.Z. J. agric. Res. 2:* 1195–202.

Rickard, D. S. 1960: The occurrence of agricultural drought at Ashburton, New Zealand. *Ibid. 3:* 431–41.

WILT AND DIEBACK OF THE CARNATION IN NEW ZEALAND

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ABSTRACT

Vascular wilt of carnation in New Zealand is commonly caused by *Fusarium oxysporum* var. *dianthi* (Prill. and Del.) Snyder and Hansen. Sometimes *Verticillium dahliae* Kleb. is responsible. *Verticillium cinerescens* Wollenw., a fungus of great importance in Europe, has been found on one property, in Christchurch, where it was introduced with cuttings imported from England in 1958. The bacterial wilt organism, *Pseudomonas carophylli* (Burkholder 1942) Starr and Burkholder 1942 was not found. *Fusarium culmorum* (W. G. Sm.) Sacc. does not produce a vascular wilt, but is important in plantings as the commonest cause of dieback and branch rot; it also causes a basal rot of young plants.

INTRODUCTION

Wilt diseases in carnation are a common cause of mortality. Most of the organisms associated are soil-borne, and may be killed by soil sterilisation. The advantage obtained by soil treatment, however, is frequently lost, since carnations are vegetatively propagated, and the wilt diseases, being systemic, are carried over in cuttings. Vascular wilts are, therefore, a major problem wherever carnations are grown.

In England Wickens (1935) showed that wilt in carnation was caused by the fungi *Fusarium dianthi* (= *F. oxysporum* var. *dianthi* (Prill. and Del.) Snyder and Hansen) and *Verticillium cinerescens* Wollenw. These two fungi are associated with carnation wilt in Denmark (Hellmers 1958), the United States of America (Nelson *et al.* 1960), and in many other parts of the world. Hellmers also recorded a bacterial wilt of carnation, caused by *Pseudomonas caryophylli* (Burkholder 1942) Starr and Burkholder 1942. The carnation was listed as a host of *Verticillium dahliae* Kleb. by Engelhard (1957).

In New Zealand Cunningham (1931) recorded *Fusarium dianthi* from nursery stock in Auckland, and Robinson (1957) recorded *Verticillium dahliae* Kleb. from the same district.

Wilted carnation plants from most districts of New Zealand have been examined in order to ascertain the importance of these fungi, and to determine whether *V. cinerescens* or bacterial wilt were present.

Cultures have been prepared from discoloured vascular tissue, from basal rots, and from dieback lesions. The organisms isolated have been identified and tested for pathogenicity.

ISOLATION OF FUNGI

With vascular wilts one of two methods of isolation was used. In the first method transverse sections were cut from the less wilted shoots, each at a node. These were rinsed in a 25 per cent solution of "Milton" (containing 1.0 per cent sodium hypochlorite). The sections were placed in Petri dishes, and plain agar, at 50°c, was poured over them. The warm agar renders ineffective any residual sodium hypochlorite. Isolates were subcultured to freshly made potato dextrose agar (PDA).

In the second method pieces of stems were surface sterilised with a 25 per cent alcoholic solution of "Milton". The stained vascular tissue was exposed with a sterile scalpel, and small pieces of this tissue were transferred to plain agar. The second method was used when the staining was prominent. In order to obtain more rapid sporulation of *Fusarium* species, isolates were transferred from agar to autoclaved pieces of carnation stems.

IDENTIFICATION OF THE FUNGI

Cultures from more than 100 carnation plants were prepared and examined. PDA cultures were examined when 3 weeks old. Cultural characters were noted, and a microscopic examination was made of conidia and mycelium. The majority of cultures could be readily identified as *Fusarium* spp. but *Verticillium* spp. were occasionally isolated.

Two species of *Fusarium*, namely *F. oxysporum* var. *dianthi* and *F. culmorum* (W.G.Sm.) Sacc., were commonly isolated. They were characterised by their relatively quick growth, pigmentation of media, and slimy sporodochia.

Fusarium oxysporum var. *dianthi* was identified by a lighter pigmentation, and free production of microconidia. Macroconidia were produced on orange-coloured sporodochia, often 1–2 mm in diameter. These macroconidia were sickle-shaped, 3–5 septate, with a distinct foot cell, and attenuated apex, 25–74μ × 3.5–4.5μ. Intercalary chlamydospores 5–12μ in diameter were freely produced in some cultures. The New Zealand isolates agreed with the description of *F. oxysporum* var. *dianthi* given by Wickens (1935) and Hellmers (1958).

In *Fusarium culmorum*, cultures were faster growing, and with darker, carmine pigmentation; while slimy sporodochia, if produced, were carmine or rust coloured. Chlamydospores were commonly formed, often intercalary and in clusters. Macroconidia were 23–50μ ×4.5–6.5μ, i.e., shorter and broader than those of *F. oxysporum* var. *dianthi*.

TABLE 1. INOCULATION STUDIES WITH ISOLATES OF FUNGI FROM CARNATION

Fungus	Number of isolates tested	Total No. of plants inoculated	No. of plants with local lesions only	No. of plants showing vascular discolouration	No. of isolations made from vascular tissues	No. of isolations yielding inoculated fungus
Fusarium oxysporum var. *dianthi*	5	78	2	71	73	71
Fusarium culmorum	3	60	23*	0	20	0
Verticillium cinerescens	1	10	0	6	6	6
Verticillium dahliae	1	10	0	4	4	4

* *F. culmorum* was re-isolated in all cases.

Two species of *Verticillium* were isolated from carnations. Both were slow-growing in culture, compared with *Fusarium* spp., and were hyaline with no reddish pigmentations.

V. cinerescens can be separated from *V. dahliae* by the absence of microsclerotia, and the presence of numerous clusters of conidiophores amongst the superficial mycelium. The hyaline, fusiform conidia, 3.5–5.5μ × 1.5–2.5μ, are budded off from the short terminal branches of the conidiophores. The New Zealand isolates agreed with the description given by Wickens (1935). Hellmers (1958) classifies this fungus as *Phialophora cinerescens* (Wollenw.) van Beyma. However, as Moore (1959) states: "It is not a good *Verticillium* because it has fasciculate conidiophores, a character which excludes it equally from the genus *Phialophora* to which van Beyma transferred it."

In *V. dahliae*, conidiophores are borne singly, verticillately branched throughout their length. Dark coloured microsclerotia were produced in the cultures from carnation. The conidia are hyaline, oval, 3–6μ × 1.5–2.5μ.

PATHOGENICITY

Potted seedling "Chabaud" carnation plants were inoculated with isolates of *Fusarium spp.* and *V. dahliae*. Cultures grown for two weeks on autoclaved carnation-stem pieces were used. A small piece of inoculum was inserted into an aseptically made cut near the base

Fig. 1.—*Left*—Healthy plant. *Right*—Staining of vascular tissue caused by *Verticillium cinerescens*. Note the absence of rotting at the base and in the cortical tissues.

TABLE 2. OCCURRENCE OF WILT AND DIEBACK IN NEW ZEALAND

Fungus	Auckland	Wellington	Christchurch	Other districts
Fusarium oxysporum var. *dianthi*	20	6	8	6
Fusarium culmorum	31	5	21	11
Verticillium cinerescens	0	0	1	0
Verticillium dahliae	4	0	1	0

The figures in the table are the numbers of disease outbreaks on growers' properties caused by the various fungi.

of the tallest stem of each plant, and the cuts covered with cellulose tape. Control plants were cut but not inoculated. The plants were held at 65°F and 100 per cent relative humidity for 24 hours, after which they were removed to a glass-house.

With *V. cinerescens*, cultures on agar were macerated and mixed with crushed pumice of a type commonly used as a rooting medium for cuttings. Cuttings of a seedling perpetual carnation were inserted in the inoculated pumice.

When plants were showing visible signs of wilting, isolations onto agar were made from vascular tissue 1 in. to 2 in. above the point of inoculation, and also from margins of local lesions. The results of the inoculation studies are summarised in Table 1.

Vascular discolouration was prominent in plants inoculated with *F. oxysporum* var. *dianthi*, *V. cinerescens*, and *V. dahliae*. With *V. cinerescens* the staining was characteristically streaky. *F. oxysporum* var. *dianthi* frequently caused retting of the cortical tissue, whereas the Verticillium species did not. (See Fig. 1).

F. culmorum caused no vascular staining, the effect being localised to a lesion near the point of inoculation. Sometimes the rot girdled the stem and caused the death of the shoots above it.

OCCURRENCE OF THE PATHOGENS IN N.Z.

Table 2 shows the distribution of the fungi associated with wilt and dieback of carnation in New Zealand. This is a summary of the isolations made from plants over a period of about six years.

F. oxysporum var. *dianthi* is the commonest cause of vascular wilt of carnation in New Zealand.

F. culmorum is very common, and widespread throughout the country. It causes considerable damage as a basal rot of young plants, and as a dieback of shoots.

V. cinerescens was present on only one property, in Christchurch, where it caused severe losses. The infection has been traced to plants of two varieties imported from England in 1958 by the grower. The plants did not produce symptoms during the post-entry quarantine period. The Department of Agriculture has since been responsible for eliminating the disease on this property.

V. dahliae was isolated on only five occasions; as appears to be the case overseas, this fungus is of no great importance to carnations in New Zealand.

The bacterial wilt organism *Pseudomonas caryophylli* was not isolated during the survey.

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REFERENCES

CUNNINGHAM, G. H. 1931: Additions to the recorded plant diseases of New Zealand. *N.Z. J. Agric. 42:* 305–6.

ENGELHARD, A. W. 1957: Host index of *Verticillium albo-atrum* Reinke and Beth. (including *Verticillium dahliae* Kleb.). *Plant Dis. Reptr. Suppl. 244:* 45.

HELLMERS, E. 1958: Four wilt diseases of perpetual flowering carnations in Denmark. *Dansk bot. Ark.: 18 (2):* 1–200.

MOORE, W. C. 1959: "British parasitic fungi." p. 424. University Press, Cambridge. 430 pp.

NELSON, P. E.; J. TAMMEN; R. BAKER 1960: Control of vascular wilt diseases of carnation by culture-indexing. *Phytopathology 50:* 356–60.

ROBINSON, J. A. 1957: Control of carnation diseases in New Zealand. *N.Z. Plants & Gdns 2:* 212–8.

WICKENS, G. M. 1935: Wilt, stem rot and dieback of the perpetual flowering carnation. *Ann. appl. Biol. 22:* 630–83.

THE ESTIMATION OF SEASONAL SOIL MOISTURE DEFICITS AND IRRIGATION REQUIREMENTS FOR ASHBURTON, NEW ZEALAND

II. Irrigation requirements

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(Received 18 December 1961)

ABSTRACT

A survey of supplemental irrigation requirements for Ashburton, New Zealand, was carried out for the September to April period of the years 1912-3 to 1955-6.

Day-by-day changes in soil moisture deficits were calculated, using daily rainfall figures and mean daily estimated evapotranspiration values for each month. The frequency and distribution of two irrigation treatments were determined. Loss of rainfall as drainage was estimated by crediting to drainage all rain which occurred while the soil was at field capacity.

It was found that where irrigation was applied to prevent the soil moisture deficit from increasing beyond 3.2 in., an average of 7.0 irrigations per season was required. The range in the number of irrigations required was from 4 to 11. The amount of drainage averaged 7.91 in., which was 38.9 per cent of the rainfall received. The average "effective" rainfall (calculated by subtracting the amount of drainage from the total rainfall) was 12.40 in., or 61.1 per cent of the total rainfall. The average seasonal soil moisture deficit which was replenished by irrigation was 9.54 in. The addition of this to the "effective" rainfall gave a total water requirement for an average season of 21.94 in.

If irrigation were applied to prevent the soil moisture deficit from increasing beyond permanent wilting point (a deficit of 2.4 in.), an average of 3 irrigations per season was required. The range in number of irrigations required per season was from 1 to 5.

INTRODUCTION

Of the 750,000 acres in Canterbury which are suitable for irrigation some 180,000 are already served by large irrigation schemes. The soils which require irrigation, and which can be suitably prepared for it, are mainly stony soils on gravels related to the yellow-grey earths (Taylor and Cox 1950). The present investigation is based on the moisture-holding characters of the Lismore stony silt loam, which is a typical and important member of this group of soils.

Before irrigation schemes are planned in detail it is important to obtain an estimate of the probable irrigation requirements of the areas concerned. Previous work in New Zealand has been confined to general surveys, e.g., those of Garnier (1951) and Gabites (1956). Any estimate of irrigation requirements which is based on the use of average monthly rainfall figures does not supply information on the probable variation from season to season. It is this variation which is of main interest to the engineer, the farm advisory officer, and the farmer. The present study has, therefore, been designed to examine in detail the probable irrigation requirements of one area. The extent to which the results obtained can be applied to other areas will depend on the variations in soil type and rainfall encountered.

The survey was based on mean daily potential evapotranspiration figures for the months September to April in the period from 1912–3 to 1955–6.

METHOD

The basic method has been described (Rickard 1960). Irrigation was assumed to be applied when the soil moisture deficit reached a pre-determined value, and two irrigation treatments were chosen—at a soil moisture deficit of 1.2 in. and 2.4 in. respectively. The former corresponds to 50 per cent available moisture in the top 12 in. of soil, and the latter to wilting point, or nil available moisture in the top 12 in. of soil. Cumulative deficits were calculated from September 1 until the appropriate deficit for the treatment was reached. An irrigation was assumed to be applied which reduced the deficit exactly to zero with no loss of irrigation water as drainage, and the calculation was recommenced on the following day. Losses of rainfall as drainage were calculated separately for each irrigation treatment by assuming that any rain which fell while the soil was at field capacity was lost as drainage.

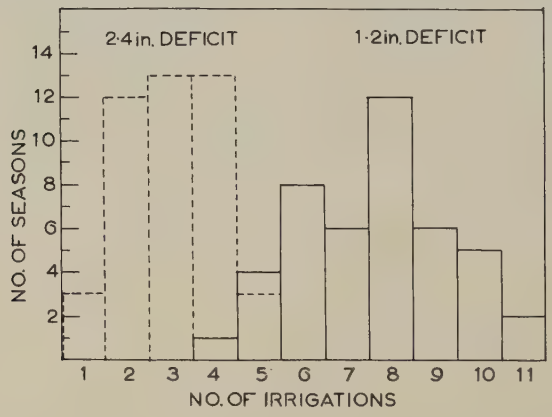

Fig. 1.—Frequency distribution for number of irrigations against number of seasons.

RESULTS

Irrigation at a soil moisture deficit of 1.2 in.

Over the 44 seasons studied there was a total of 336 irrigations, an average of 7.6 per season. The greatest number of irrigations required in any season was 11 (in 1916–7 and 1954–5), and the least number was 4 (1944–5). It is of interest to note that, even in the wettest season, four irrigations were required to prevent the soil moisture deficit from increasing beyond 1.2 in. The frequency distribution for number of irrigations against number of seasons is given in Fig. 1. From this graph, it is possible to estimate the percentage of seasons during which a certain number of irrigations would be required. For example, if it is practicable on a particular irrigation scheme to apply up to eight irrigations at a soil moisture deficit of 1.2 in., then approximately 70 per cent of the seasons studied would be included. If up to nine irrigations could be accommodated, then 84 per cent of the seasons would be included. This may be taken as a broad indication only : the limiting factor governing irrigation frequency in a particlar season is likely to be the interval between the times when irrigation can be applied. (See below.)

TABLE 1. AVERAGE DISTRIBUTION OF IRRIGATION THROUGHOUT THE SEASON

Month	Total number of irrigations	As percentage of the total
September	1	0.3
October	22	6.6
November	44	13.1
December	68	20.2
January	69	20.5
February	60	17.9
March	53	15.8
April	19	5.7
Season	336	100.1

The average distribution of irrigations throughout the season is given in Table 1. The fact that only once in 44 seasons was an irrigation required in September should be subject to the following qualification : in carrying out the survey, the assumption was made that the soil would be at field capacity on September 1, and although this is generally correct, a particularly dry August can result in the soil being slightly below field capacity on that date. Had it been possible to take this fact into consideration, it is probable that there would have been more irrigations during September, although they would all be concentrated towards the end of the month.

The time interval between successive irrigations is a more important factor in the design and operation of irrigation schemes than the total number of irrigations per season. This is true not

only for large scale schemes serving a number of farmers, but also for small schemes covering only one farm. The length of the interval between irrigations will depend on the distribution of rainfall and on the variation of the daily evapotranspiration value throughout the season. Fig. 2 shows the intervals between irrigations at a soil moisture deficit of 1.2 in. expressed as a percentage of the total number of irrigations. In no case was the interval less than 5 days, and intervals of between 5 and 10 days make up only approximately 6 per cent of the total number. It is apparent, therefore, that for practical purposes periods of less than 10 days could be ignored. Nearly half (48.9 per cent) of all periods between irrigations were between 11 and 20 days in duration.

Where irrigation is applied, it is to be expected that the loss of rainfall by drainage through the soil would be increased. Frequently irrigation is followed within a few days by rain, much of which cannot be retained by the moist soil. In the present study the assumption was made that each irrigation exactly replaced the soil moisture deficit; there was, therefore, no loss of irrigation water by drainage.

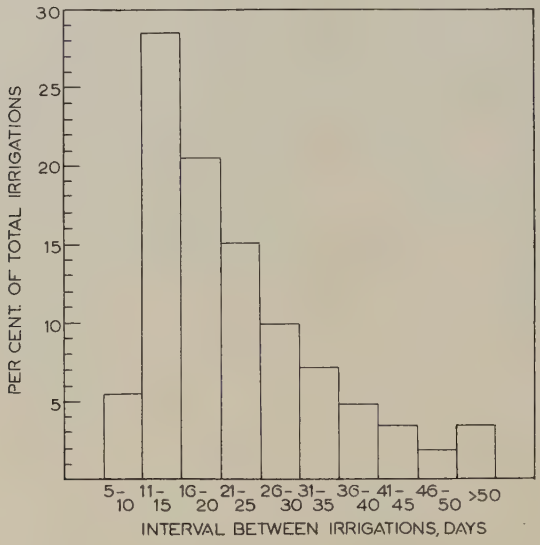

Fig. 2.—Intervals beetween irrigations applied at a deficit of 1.2 in.

Under soil conditions where irrigation was applied at a soil moisture deficit of 1.2 in., the average loss of rainfall by drainage per season was calculated to be 7.90 in., compared with 4.50 in. previously reported for a non-irrigated area (Rickard 1960). This represents a 75 per cent increase in drainage loss. (It is an unfortunate fact that the application of irrigation water decreases the amount of effective rainfall.) The average rainfall per season

over the years of the survey was 20.30 in., and the effective rainfall was 12.40 in. (i.e., 20.30 in. less 7.90 in. drainage), or 61.1 per cent of the average.

During a season the total amount of water used by a pasture can be assumed to be supplied from only two sources—rainfall and irrigation. The amount of irrigation water retained by the soil in any one season is obtained from the total of the soil moisture deficits existing just prior to each irrigation. Although the level of irrigation had been fixed at a 1.2 in. deficit, at each irrigation the actual deficit will be slightly higher than this—depending on the daily evapotranspiration rate at the particular time of the season. For example, during 1955–6 irrigation was carried out eight times. The dates of, and deficits at, each irrigation were as follows:

Date	Deficit (in.)
1.11.55	1.25
18.11.55	1.21
9.12.55	1.30
21.12.55	1.32
5.1.56	1.31
16.1.56	1.31
14.2.56	1.27
29.2.56	1.22
Total deficit: 10.19 in.	

If the exact amount of irrigation water necessary to replace that lost by evapotranspiration was applied at each irrigation, the total for the 1955–6 season would be 10.19 in. This total was calculated for each season, and averaged 9.54 in., ranging from 4.97 in. (4 irrigations) to 13.90 in. (11 irrigations). The total amount of water lost by evapotranspiration from a pasture in an average season will therefore be 12.40 in. (effective rainfall), plus 9.54 in. (irrigation), that is, a total of 21.94 in.

Rainfall, drainage, total deficit, and water requirement figures for each of the 44 seasons are given in Table 2.

Irrigation at the 2.4 in. deficit

This irrigation treatment is applied when the soil reaches permanent wilting percentage—a deficit of 2.4 in. for the top 12 in. of soil. Where conditions of acute water shortage exist, it may be necessary to design and operate an irrigation scheme based on the minimum usage of water. This would entail irrigating at the above deficit, and data for such an irrigation were obtained along the same lines as for the 1.2 in. deficit irrigation treatment.

For the 44 seasons studied there was a total of 133 irrigations, an average of 3 per season. The greatest number in any season was 5 (occurring in the seasons 1914–5, 1923–4, and 1940–1), and the least number, one (1922–3, 1944–5, and 1950–1). It should be noted that, even in the wettest seasons, a deficit of 2.4 in., and hence an irrigation, was reached at least once.

TABLE 2. HYDROLOGICAL CYCLE UNDER TWO IRRIGATION TREATMENTS
SEASONS 1912–3 TO 1955–6

Season	Rain	1.2 in. deficit treatment				2.4 in. deficit treatment			
		Drainage	Effective rain	Total deficit	Water requirement	Drainage	Effective rain	Total deficit	Water requirement
1912–13	19.74	7.16	12.58	8.69	21.27	6.63	13.11	7.44	20.55
–14	24.31	9.79	14.52	7.44	21.96	7.35	16.96	4.93	21.89
–15	11.74	2.24	9.50	12.48	21.98	2.57	9.17	12.27	21.44
–16	13.86	2.40	11.46	10.18	21.64	0.58	13.28	7.23	20.51
–17	13.32	4.98	8.34	13.90	22.24	1.09	12.23	9.79	22.02
–18	22.74	8.59	14.15	7.45	21.60	6.81	15.93	4.89	20.82
–19	17.14	6.61	10.53	11.27	21.80	8.15	11.99	9.85	21.84
–20	21.69	9.17	12.52	9.77	22.29	6.69	15.00	7.44	22.44
–21	21.59	9.25	12.34	9.92	22.26	7.46	14.13	7.43	21.56
–22	22.70	10.68	12.02	10.02	22.04	9.30	13.40	7.36	20.76
–23	19.58	4.29	15.29	7.45	22.74	0.75	18.83	2.41	21.24
–24	13.25	3.38	9.87	12.39	22.26	2.83	10.42	12.12	22.54
–25	21.56	8.32	13.24	8.74	21.98	7.41	14.15	7.38	21.53
–26	18.66	7.88	10.78	11.37	22.15	6.26	12.40	9.77	22.17
–27	17.24	2.82	14.42	7.60	22.02	2.58	14.66	7.35	22.01
–28	18.32	3.76	14.56	7.57	22.13	2.68	15.64	4.88	20.52
–29	21.75	7.00	14.75	7.40	22.15	4.51	17.24	4.90	22.14
–30	17.55	4.71	12.84	8.78	21.62	3.22	14.33	7.29	21.62
–31	18.57	7.18	11.39	10.09	21.48	4.50	14.07	7.40	21.47
–32	14.90	5.55	9.35	12.62	21.97	4.11	10.79	9.77	20.56
–33	14.88	5.51	9.37	12.43	21.80	1.98	12.90	7.37	20.27
–34	24.68	8.94	15.74	6.15	21.89	7.93	16.75	4.84	21.59
–35	16.77	4.72	12.05	10.01	22.06	4.53	12.24	9.81	22.05
–36	30.98	16.59	14.39	8.74	23.13	13.81	17.17	4.96	22.13

–37	23.31	7.97	15.34	7.46	22.80	6.12	17.19	4.92	22.11
–38	24.97	11.50	13.47	8.61	22.08	8.93	16.04	4.95	20.99
–39	19.08	8.34	10.74	11.12	21.86	6.35	12.73	9.97	22.70
–40	15.83	4.98	10.85	11.31	22.16	3.25	12.58	9.76	22.34
–41	25.78	17.22	8.56	12.55	21.11	16.74	9.04	12.19	21.23
–42	15.62	5.51	10.11	11.19	21.30	1.16	14.46	4.90	19.36
–43	18.21	4.49	13.72	8.67	22.39	3.49	14.72	7.33	22.05
–44	22.88	8.16	14.72	9.91	24.63	8.04	14.84	9.79	24.63
–45	33.53	16.67	16.86	4.97	21.83	14.86	18.67	2.51	21.18
–46	16.78	5.49	11.29	10.15	21.44	5.21	11.57	9.63	21.20
–47	31.09	17.56	13.53	6.24	19.77	13.47	17.62	4.82	22.44
–48	17.40	5.39	12.01	9.96	21.97	5.15	12.25	9.68	21.93
–49	16.99	5.77	11.22	11.11	22.33	4.51	12.48	9.77	22.25
–50	23.42	9.01	14.41	7.47	21.88	6.90	16.52	4.85	21.37
–51	32.35	15.61	16.74	6.15	22.89	13.07	19.28	2.41	21.69
–52	15.96	5.34	10.62	9.93	20.55	2.23	13.73	7.33	21.06
–53	36.28	20.81	15.47	6.26	21.73	19.50	16.78	4.89	21.67
–54	16.37	5.19	11.18	10.09	21.27	2.80	13.57	7.37	20.94
–55	14.43	6.79	7.64	13.74	21.38	3.26	11.17	9.72	20.89
–56	15.40	4.13	11.27	10.19	21.46	3.84	11.56	9.89	21.45
Mean	20.30	7.90	12.40	9.54	21.94	6.13	14.17	7.40	21.57

The frequency distribution for number of irregations against number of seasons is given in Fig. 1. No irrigations were required in September or October, and 62.4 per cent of all irrigations occurred in the three months December–February. The interval between irrigations ranged in length upwards from 20 days, slightly more than half the irrigation intervals being less than 40 days.

Drainage losses were calculated in the same way as for the previous irrigation treatment. The range was from 0.58 in. to 19.50 in., with a mean value of 6.13 in. The mean seasonal effective rainfall was, therefore, 14.17 in. The total seasonal deficit was calculated in the same way as previously, and ranged from 2.41 in. (one irrigation) to 12.57 in. (5 irrigations), with a mean value of 7.40 in. The total water requirement under this system was 21.57 in., made up of 14.17 in. (contribution from rainfall) plus 7.40 in. (contribution from irrigation). Drainage, effective rainfall, total deficit, and water requirement figures for each of the 44 seasons are given in Table 2.

The various contributions of rainfall, drainage, and irrigation to the hydrological cycle are shown in Table 3. The figures relating to non-irrigated areas have been taken from Rickard (1960).

TABLE 3. SEASONAL WATER BALANCE UNDER IRRIGATED AND NON-IRRIGATED CONDITIONS

	Non-irrigated	Irrigated at deficit of 2.4 in.	Irrigated at deficit of 1.2 in.
(a) Rainfall (in.)	20.30	20.30	20.30
(b) Drainage (in.)	4.50	6.13	7.90
(c) Effective rainfall (in.) (a–b)	15.80	14.17	12.40
(d) Applied by irrigation (in.)	—	7.40	9.54
(e) (Total water used (c + d)	15.80	21.57	21.94
(f) Number of irrigations	—	3	7–8

DISCUSSION

By selecting deficits of 1.2 in. and 2.4 in., a comparison is obtained between a relatively intensive system of irrigation and a system which could be regarded as a minimum usage of irrigation water. These two irrigation treatments form the basis of a pasture production experiment at Winchmore Irrigation Research Station, Ashburton, and from the results obtained over a number of seasons it seems probable that irrigation at a deficit of 1.8 in.—corresponding to 25 per cent available soil moisture—may reduce the number of irrigations required and hence the labour and water demand, and still provide a reasonable and non-fluctuating level of pasture production. From Table 3 it is possible to inter-

polate values for such a treatment. The following results were obtained:

Drainage	7.0 in.
Effective rainfall	13.3 in.
Applied by irrigations	8.7 in.
Total water used	22.0 in.
Number of irrigations	4–5

There are many factors which affect the practice of irrigation —both at the planning and construction stages—and the day-by-day operation on the farm. Engineering construction details in large schemes of border dyke irrigation, and the provision of spray equipment within certain financial limits on smaller schemes, will have an effect on the ultimate availability of water, and it is obviously not practicable for water to be available at each paddock exactly as required. From the farmer's point of view, there are other considerations—such as competing labour requirements, stock management, and the particular water requirements of different crops—which make it difficult or even inadvisable to irrigate strictly to a soil moisture deficit basis. The information contained in the present study, therefore, should be considered in the light of these other factors.

It is of interest to note the changes in the hydrological cycle brought about by irrigation. Under an intensive irrigation system drainage is increased from 22.2 per cent to 38.9 per cent of the rainfall, and the effective rainfall is reduced to 61.1 per cent of the total seasonal rainfall. Under non-irrigated conditions the effective rainfall is approximately 6 in. less than the seasonal water requirement.

REFERENCES

Gabites, J. F. 1956: The estimation of natural evaporation and water need. *Proc. Conf. Soil Moisture N.Z Dep. sci. industr. Res. Inf. Ser. 12:* 122–31.

Garnier, B. J. 1951: The application of the concept of potential evapotranspiration to moisture problems in New Zealand. *N.Z. Geogr. 7:* 43–61.

Rickard, D. S. 1960: The estimation of seasonal soil moisture deficits and irrigation requirements for Ashburton, New Zealand. I. Soil moisture deficits. *N.Z. J. agric. Res. 3:* 820–8.

Taylor, N. H.; Cox, J. E. 1956: The soil pattern of New Zealand. *Proc. N.Z. Inst. Agric. Sci.* 28–44.

DRY-ROT (*Leptosphaeria maculans*) OF BRASSICAS: SEED TRANSMISSION AND TREATMENT

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ABSTRACT

The extent of seed transmission of dry-rot was assessed over a period of three years by sowing seed lines with known levels of infection and noting the amount of dry-rot which subsequently developed in each line. If weather conditions were wet after sowing, transmission of the disease occurred from seed with an infection of 0.5 per cent; but in a dry season a level as high as 3.0 per cent gave no transmission. As the infection in most commercial seed sown in New Zealand has been shown rarely to exceed 0.1 per cent, it is concluded that the annual recurrence of this disease in swede-growing areas is not caused by seed infection. This is regarded as strong circumstantial evidence that air-borne ascospores originating from old brassica crop residues are the chief agents of primary infection in the majority of swede crops in New Zealand.

Laboratory trials showed that infection of brassica seed by *Leptosphaeria maculans* (Desm.) Ces. & de Not. (*Phoma lingam*) could be greatly reduced by seed treatment with several fungicides. Seed treatment gave no control of dry-rot in field trials, however, except in cases where artificially inoculated seed was used.

INTRODUCTION

It was thought at one time (Cunningham 1927) that control of seed-borne infection would eventually lead to complete elimination of the dry-rot disease of brassicas, and efforts to produce seed free from infection led to the first large-scale commercial production of swede and turnip seed in New Zealand. Since 1940 New Zealand has produced most of her own requirements of swede, turnip, and rape seeds, the bulk of this production being organised by the Department of Agriculture and distributed through merchants as "certified" or "Government Approved" seed. Such seed has been regularly tested for dry-rot infection and found to be completely free in most years. When present, the level of the disease in the seed rarely exceeds 0.1 per cent (Lloyd 1959), and is usually much lower. In spite of this, dry-rot has remained the most serious disease of swedes in the main growing areas of Otago and Southland.

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The discovery of the *Leptosphaeria* stage of the dry-rot fungus (Smith 1956, 1960) provided an answer to this problem, as it was obvious that the air-borne ascospores were capable of causing infection in crops otherwise free of the disease. Moreover, surveys showed that the perithecial stage of *Leptosphaeria maculans* (Desm.) Ces. & de Not. (*Phoma lingam*) was common on residues from swede, turnip, chou moellier (marrow-stem kale), rape, and kale crops, and it was thought that the wind-borne ascospores constituted the main source of infection for swede and turnip crops in Canterbury, Otago, and Southland. The relative significance of seed-borne and wind-borne infection was not known, however, and trials were carried out to establish the extent to which the disease was transmitted by seed. In the field trials described below most of the figures relating to seed infection are based on samples of 20,000 seed, although samples of 1,000 were used for inoculated seed, and 60,000 seed of the "dry-rot free" line were tested.

TRANSMISSION BY SEED

The extent of transmission of disease by seed was assessed by comparing the number of infections arising in plants grown from seed free of dry-rot with the number appearing in lines with different known levels of seed infection. This was first done in a trial laid down in the 1957-8 season at Oreti (Southland), in which four seed lines of chou moellier with different levels of dry-rot infection were sown in two-row strips between six-row bocks of swedes. The main object of this trial was to assess the importance of chou moellier seed as a source of inoculum for spreading dry-rot in swede crops sown with chou moellier. The level of dry-rot infection in the seed was determined using the 2, 4-D germination technique (Lloyd 1959). The swede seed was free from dry-rot infection, and the chou moellier seed lines consisted of:

1. Healthy seed, treated with hot water and dusted with Ceresan.
2. Naturally infected seed treated with hot water and dusted.
3. Naturally infected seed untreated.
4. Inoculated seed.

TABLE 1. SEED TRANSMISSION TRIAL 1958
Percentage total infection in chou moellier stems and swede bulbs

Treatment	Chou moellier seed infection %	Chou moellier		Swede	
		3 months	9 months	7 months	9 months
1	Nil	0	0	7	18
2	0.06	0	0.05	11	21
3	0.5	0.5	0.5	17	28b
4	15.0	15.0a	20.0a	32a	37a

a = significantly different from treatments 1, 2, and 3 (P = 0.05)
b = significantly different from treatments 1 and 2 (P = 0.05)

Counts of dry-rot (Black Leg) infection in the chou moellier plants three months and nine months after sowing and of dry-rot in swedes adjacent to the chou moellier rows seven months and nine months after sowing are shown in Table 1.

These results indicate that a natural infection of 0.5 per cent in chou moellier seed may give rise to infected plants in the field.

A trial with swedes was laid down in 1958–9 at Gore, in which the following six seed lines were sown (the varieties Grandmaster and Superlative being considered equally susceptible to dry-rot by Cruickshank and Palmer 1954):

1. N.Z. Grandmaster : dry-rot free.
2. „ : 0.02 per cent infection.
3. „ : 0.05 per cent infection.
4. „ : 0.1 per cent infection.
5. N.Z. Superlative : 0.3 per cent infection.
6. „ : 3.0 per cent infection (inoculated seed).

These were sown in four replications of a randomised block layout, each plot consisting of 18 rows two chains in length in the case of lines 1–3, six rows in the case of lines 4 and 5, and two rows in the case of the inoculated line 6. In order to discount as far as possible the effects of air-borne infection and secondary spread, only severely infected (i.e. early infected) bulbs were counted in this year. A count made seven months after sowing gave the results shown in Table 2.

TABLE 2. SEED TRANSMISSION TRIAL 1959
Percentage severe infection in swede bulbs

Seed infection %	Bulb infection %
Nil	1.0
0.02	1.1
0.05	1.2
0.1	1.1
0.3	0.9
3.0	1.4

The correlation between seed and bulb infection is not significant.

In the 1959–60 season, two similar trials were carried out at Gore and Clinton using seed of the variety N.Z. Grandmaster.

In each case six replicates were sown in a randomised block layout. The plots were one chain long, and consisted of six rows in the case of seed line 3, and eighteen rows in the case of lines 1 and 2. The results of a count of severely affected bulbs made five months later are shown in Table 3.

Fig. 1.—Dry-rot infection of cotyledons of inoculated chou moellier seed germinated in soil in humidity cabinet.

TABLE 3. SEED TRANSMISSION TRIAL 1960
Percentage severe infection in swede bulbs

Line	Seed infection %	Bulb infection %	
		Gore	Clinton
1	Nil	5.0	3.1
2	0.07	6.0	2.9
3	0.5	5.6	7.0**

At Clinton the bulb infection in line 3 was significantly greater ($P = 0.01$) than in the other lines. At Gore there were no significant differences.

SEED TREATMENT STUDIES

Preliminary laboratory tests were carried out to determine the relative efficacy of a range of fungicides in controlling the seed-borne infection by *L. maculans*. As it was well-nigh impossible to carry out this work in the absence of a highly infected line of seed, it was necessary to use artificially inoculated seed. Since none of the methods of

inoculation previously described in the literature was entirely satisfactory, the technique finally adopted was developed in the laboratory of Plant Diseases Division, D.S.I.R., Lincoln, and was subsequently used by Lloyd (1959). It involved spraying washed seed with a pycnospore suspension of *L. maculans,* incubation under conditions suitable for the growth of the fungus, and then drying quickly to prevent sprouting of the seed. Seed inoculated in this way was found to germinate satisfactorily even when slight sprouting did take place, and the degree of infection obtained was generally high, varying from 20 per cent to 100 per cent when tested either on agar plates or germinated under high humidity. (See Fig. 1.).

The method of seed treatment used in the laboratory trials with inoculated seed was to add known quantities of the dusts or liquids to 10g samples of brassica seed and shake for 5 min in a laboratory shaker. From each sample five lots of 100 seeds were then plated on prune agar, to which had been added 2,000 p.p.m. acid equivalent of the sodium salt of 2,4-D (Lloyd *loc. cit.*): The 2,4-D prevented normal germination of the seed and thus made examination of the seed easier. Examination for the presence of *L. maculans* was made after the plates had been incubated for 14 days at 25°c. A later modification of this technique for testing large numbers of seeds was to germinate them on thick absorbent paper impregnated with 0.2 per cent 2,4-D solution. (See Fig. 2.).

Laboratory trials using inoculated seed showed that captan and organic mercury preparations were superior to thiram and quinone materials in their control of the dry-rot fungus, and the fungicides eventually chosen for testing in the field during the 1958–9 season were Ceresan, Orthocide 75, a 50:50 mixture of Ceresan and Orthocide 75, and Panogen. Each was used at rates of 0.05 per cent, 0.1 per cent, and 0.2 per cent of the commercial formulation, giving a total of 15 treatments, including checks. These were sown in a randomised block layout, each plot consisting of four rows, one chain in length.

Three trials were laid down in the Otago/Southland area: at Invermay, Winton, and Otama. At Invermay four replications were sown, using N.Z. Calder Swede seed with a dry-rot infection of 0.4 per cent. At Winton eight replications were sown, four with N.Z. Superlative swede seed showing 0.1 per cent infection, and four with inoculated seed of the same line with 6.1 per cent infection. The same Superlative line was used at Otama, but here only three replications of the naturally infected and two of the inoculated seed were sown.

When a count of infected bulbs was made seven months after sowing conditions appeared excellent for the demonstration of treatment differences, for in each case there was a low incidence of dry-rot throughout the trial area, indicating that little secondary spread had taken place. In order further to minimise the masking effect of secondary spread on primary infection, only severely affected bulbs were counted. A summary of the results of these counts is given in Table 4.

Fig. 2.—Dry-rot infection of seed tested on 2, 4-D soaked filter paper.

In the case of the inoculated seed at Winton and Otama the checks are significantly higher than all other treatments at the 5 per cent level. There are no other differences. The control of infection in the inoculated seed, only, showed that there was little or no transmission to be controlled in the seed lines with 0.4 per cent and 0.1 per cent infection.

A further trial carried out at Otama provided further evidence that control of seed infection need give no control of dry-rot. Three rows, eight chains in length, of 16 different swede lines, each of which had been previously tested for dry-rot in the laboratory, were sown. One row of each line was sown with untreated seed, one with hot water-treated seed, and the third with Ceresan-treated seed. The hot water treatment, in which the seed was immersed for 25 min at 50°c, was included because many workers have reported this to be the only satisfactory method of eliminating dry-rot infection in seed (e.g., Walker 1922, Dennis 1941, Western 1945). A count of severely infected bulbs was made seven months after sowing, with the results shown in Table 5.

It can be seen that neither of the seed treatments gave any control of the disease and that the level of seed infection bore no relation to the incidence of primary dry-rot in the crop. The latter

TABLE 4. SEED TREATMENT TRIALS
Percentage primary infection in swede bulbs

Treatment	Rate (%)	Location				
		Invermay	Winton		Otama	
		Seed infection (%)				
		0.4	0.1	6.1	0.1	6.1
Check	—	0.7	0.7	1.6*	2.0	16.2*
Ceresan	0.05	0.5	0.7	0.1	1.3	2.2
	0.1	0.5	0.3	0.1	1.8	3.3
	0.2	1.0	0.3	0.3	1.7	5.8
Orthocide	0.05	0.3	0.1	0.4	1.8	3.2
	0.1	0.3	0.2	0.4	2.3	4.8
	0.2	0.1	0.4	0.4	2.1	4.0
Ceresan + Orthocide	0.05	0.4	0.3	0.3	1.9	2.5
	0.1	0.4	0.3	0.3	1.8	2.3
	0.2	0.5	0.4	0.3	1.1	2.2
Panogen	0.05	0.2	0.5	0.3	0.9	2.3
	0.1	0.5	0.4	0.8	1.3	1.3
	0.2	0.2	0.5	0.1	2.4	1.8

* Infection significantly higher ($P = 0.05$)

point was confirmed by testing the correlation between the figures for seed infection and those for infection in the check rows. This was not significant.

DISCUSSION

The results of the seed transmission trials show the difficulty of determining the exact level at which seed infection does become of consequence. In 1958 a level of 0.5 per cent in chou moellier gave more dry-rot in the field than disease-free seed. Although this difference was not statistically significant, the dry-rot incidence in swedes adjacent to the chou moellier lines was significantly different. It seems likely, therefore that had swede seed with an infection of 0.5 per cent been sown, this level would have been sufficient in that season to cause an appreciable increase of dry-rot in the resulting plants.

This was certainly the case in the 1960 trial at Clinton, where swede seed with an infection of 0.5 per cent gave rise to plants

with over twice as much dry-rot as did healthy seed. In the 1959 trial, however, dry-rot incidence in swedes grown from seed with infection levels as high as 3.0 per cent did not differ from that in plants grown from healthy seed.

It is possible that climatic conditions influence the extent of seed transmission in the same way as they affect the secondary spread of the disease. This was indicated by the results of unreplicated glass-house trials in which inoculated chou moellier seed with a dry-rot infection of 100 per cent was germinated under conditions of high and low atmospheric humidity. All seedlings kept at a relative humidity of 90–100 per cent developed dry-rot, whereas those kept under dry conditions remained free of the disease. (See Fig. 1.). The meteorological records of the trial areas are inadequate to support the hypothesis that humidity during emergence of the seedlings is correlated with the extent of seed transmission of dry-rot. It may be noted, however, that the highest rainfall for the month of sowing was recorded in the 1958 trial and in the 1960 trial at Clinton, and that these were the only trials in which there was evidence of transmission of dry-rot from seed with an infection level as low as 0.5 per cent.

TABLE 5. SEED TREATMENT TRIAL, OTAMA
Percentage primary infection in swede bulbs

Swede variety	Natural seed infection % before treatment	Check	Hot water-treated	Ceresan 0.2%
N.Z. Grandmaster	Nil	1.8	1.5	1.8
N.Z. Grandmaster	Nil	1.5	1.5	1.0
N.Z. Grandmaster	Nil	1.8	1.3	2.0
N.Z. Grandmaster	Nil	1.5	1.0	2.1
N.Z. Sensation	0.02	0.4	0.8	1.2
N.Z. Superlative	0.04	0.2	1.0	1.8
N.Z. Calder	0.08	1.2	0.9	1.2
N.Z. Wilhelmsburger	0.08	1.0	0.7	0.9
N.Z. Superlative	0.14	1.5	1.0	2.6
N.Z. Superlative	0.16	1.5	1.5	2.0
N.Z. Grandmaster	0.20	1.6	1.6	1.7
N.Z. Calder	0.24	0.5	0.6	0.8
N.Z. Superlative	0.25	0.8	1.5	1.5
N.Z. Superlative	0.25	0.9	0.8	1.8
N.Z. Calder	0.30	1.3	1.6	1.0
N.Z. Crimson King	0.40	1.2	0.2	1.0
Average		1.2	1.1	1.5

This hypothesis might also explain the conflicting results obtained by previous workers on the importance of seed transmission. Hughes (1933), for instance, found that seed infection bore no relation to the amount of dry-rot which developed in the crop, whereas Dennis (1941) reported that in his trials loss from dry-rot was directly traceable to seed-borne infection.

Several workers have shown that hot water treatment will control *L. maculans* on brassica seed (Walker 1922, Clayton 1927, Dennis 1941, Millard 1945, Western 1945). However, there is conflicting evidence on the value of fungicidal seed dressings. Dennis (1941), for instance, found that on swede seed Ceresan gave only a limited measure of disease control, whereas Darpoux *et al.* (1957) reported excellent control of the fungus on Colza seed by organic mercury dusts and preparations containing thiram, captan, and quinone.

The present work, on swede, turnip, and chou moellier seed, confirms that many of the seed dusts commonly used will give good field control of *L. maculans* on inoculated seed. As low levels of seed infection do not appear to be important in the transmission of dry-rot, complete elimination of *L. maculans* is not necessary, and the degree of control obtained by dusting should be sufficient to prevent any spread of the disease by infected seed.

It must be stressed, however, that in all reported instances where transmission of dry-rot by infected seed had been demonstrated, and in all cases where control of the disease has been obtained by seed treatment, the level of seed infection has been much higher than that encountered in commercial lines of New Zealand-grown brassica seed. For this reason it is concluded that even in seasons favourable to the spread of the disease by seed, transmission by this means is not likely to be important. Most swede crops in New Zealand are grown in ground out of pasture, and as the only other known means by which the disease can originate in such crops is by ascospores blown from brassica crop residues, it is further concluded that this is the chief method by which infection arises.

ACKNOWLEDGMENTS

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REFERENCES

Clayton, E. E. 1927: Black leg disease of Brussels sprouts, cabbage and cauliflower. *N.Y. (Geneva) Agric. Exp. Sta. Bull. 550.* 15 pp.

Cruikshank, I. A. M.; Palmer, T. P. 1954: Resistance of swede varieties to dry-rot (*Phoma lingam* (Tode) Desm.) *N.Z. J. Sci. Tech. A36:* 122–8.

Cunningham, G. H. 1927: Dry-rot of swedes and turnips: its cause and control. *N.Z. Dep. Agric. Bull. 133.* 51 pp.

Darpoux, H.; Louvet, J.; Ponchet, J. 1957: Essais de traitement des semences de cruciferes contre le *Phoma lingam* (Tode) Desm. et l'*Alternaria brassicae* (Berk.) Sacc. *Ann. Epiphyt. 4:* 545–57.

Dennis, R. W. G. 1941: Dry rot of swedes. The importance of seed-borne infection. *Scot. Fmr.:* 332–5.

Hughes, W. 1933: A study of *Phoma lingam* (Tode) Desm., and of the "dry-rot" it causes, particularly in swede turnips. *Sci. Proc. R. Dublin Soc. n.s. 20:* 495–530.

LLOYD, A. B. 1959: The transmission of *Phoma lingam* (Tode) Desm. in the seeds of swede, turnip, chou moellier, rape and kale. *N.Z. J. agric. Res. 2:* 649–58.

MILLARD, W. A. 1945: Canker and mosaic of broccoli, *J. Minist. Agric. 52:* 39–42.

SMITH, H. C. 1956: *Leptosphaeria napi*, the perithecial form of *Phoma lingam* causing dry-rot disease of brassicas. *N.Z. Sci. Rev. 14:* 116–7.

———1960: Control of swede dry-rot (*Leptosphaeria maculans* (Desm.) Ces. & de Not.) *Proc. N.Z. Inst. agric. Sci.:* 90–101.

WALKER, J. C. 1922: Seed treatment and rainfall in relation to the control of cabbage black leg. *U.S. Dep. Agric. Bull. 1029.* 26 pp.

WESTERN, J. H. 1945: Seed-borne fungi. *Nature, Lond. 155:* 36–7.

NITROGEN AND GRASSLAND PRODUCTION IN THE MID-ALTITUDE ZONE OF CANTERBURY, NEW ZEALAND

I. The effects of different levels of nitrogen fertiliser on herbage and nitrogen yields of cultivated pastures

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ABSTRACT

Experiments are reported showing effects of nitrogen fertilisers in increasing grassland dry matter daily increment up to levels of 150 lb per acre and annual production up to levels of more than 11,000 lb DM per acre for a wet growing season of less than five months.

The largest increases in herbage production attributable to nitrogen were obtained during the early summer period of peak growth.

Yields of total herbage, of grass, and of nitrogen in grass were shown to be functions of fertiliser nitrogen, and grass nitrogen was negatively correlated with the yield of clover nitrogen.

The significance of these findings is briefly discussed in the light of earlier conclusions concerning the relation of clover nitrogen, grass nitrogen, and fertiliser nitrogen, and also in terms of grassland management in such climatic zones.

INTRODUCTION

The mid-altitude zone of Canterbury has been described by O'Connor (1959) from the viewpoint of plant production in difficult environments. Attempts at improvement of tussock grasslands in that zone in the last decade have taken two principal forms: (a) the oversowing or sod-seeding of rough pastures of resident species with clovers, sometimes with grasses such as cocksfoot, generally accompanied by top-dressings of sulphur and phosphatic and molybdic manures; and (b) the replacement of existing pastures by sowing highly productive species with appropriate manures after cultivation. Pastures developed by cultivation at the Broken River Experimental Area in Waimakariri Catchment at 2,350 ft altitude have been described by Dingwall (1956). Some use of nitrogenous fertilisers had been made in the establishment of these pastures and in the correction of early nitrogen deficiency. With

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these cultivated pastures, as well as with the clover-oversown grasslands, the intention has been to relieve the all-pervading nitrogen dificiency by encouraging legume growth and nitrogen fixation, but this process may require many years before maximum yield can be obtained.

The present series of experiments was therefore designed to obtain quickly agronomic evidence of the productivity of cultivated pastures in this district when the supply of available nitrogen was improved by increments of fertiliser nitrogen. A second object was to determine the influence of increased nitrogen supply on spring growth rates of cultivated pastures.

EXPERIMENTAL

Pastures

The experiments were carried out on the Broken River Experimental Area of the Department of Agriculture on Craigieburn fine sandy loam. The area had been until 1954 in unimproved pastures, principally *Agrostis tenuis* Sibth., *Anthoxanthum odoratum* L., *Festuca novae-zelandiae* (Hack.) Cockayne, *Poa colensoi* Hook. f., *Cyathodes fraseri* (A. Cunn.) Allan, *Raoulia subsericea* Hook. f., and *Hypochaeris radicata* L. This poor grassland was ploughed in autumn 1954, and fallowed during the winter. The area of Experiment 1 was first used for two successive greenfeed crops, then sown on 15.3.56 to a mixture of perennial ryegrass (*Lolium perenne* L.) 15 lb, short-rotation ryegrass (derived from *L. perenne* × *L. multiflorum*) 5 lb, cocksfoot (*Dactylis glomerata* L.) 5 lb, tall oat grass (*Arrhenatherum elatius* (L.) Beauv. ex J. et C. Presl) 5 lb, timothy (*Phleum pratense* L.) 3 lb, Montgomery red clover (*Trifolium pratense* L.) 1 lb, alsike clover (*Trifolium hybridum* L.) 1 lb, white clover (*Trifolium repens* L.) 2 lb, and sheep's burnet (*Poterium sanguisorba* L.) 3 lb. In the three years before this experiment was commenced the area had received the following total per acre dressing of fertiliser: 45 cwt of lime, 9.3 cwt of superphosphate, and 50 lb nitrogen. During this period it had provided 2,490 sheep-grazing days per acre, of which 1,290 were in its 80 weeks of pasture life.

The area of Experiment 2 was sown in turnips in early summer 1954, followed by greenfeed oats in spring 1955. A pasture mixture similar to that used for Experiment 1 was drilled in spring 1956. A total amount of top-dressing similar to that of Experiment 1 had been applied to the area, and it had provided a total of 1,450 sheep-grazing days per acre. Of these 970 were in its 50 weeks of pasture life before the beginning of this experiment. On the area of Experiment 3 a temporary pasture had been sown in spring 1954 with Italian ryegrass (*Lolium multiflorum* Lam.) 30 lb, broad red clover (*Trifolium pratense*) 4 lb, and white clover 1 lb. Greenfeed oats and rape had followed in 1955–6, and permanent pasture of the same mixture as above was drilled on 17.11.56. The area had received as top-dressings a total of 45 cwt of lime, 11 cwt of superphosphate, and 50 lb nitrogen. In the four years of development up to the commencement of the experiment the area had provided approximately 4,000

sheep-grazing days per acre, of which about 2,500 were in its 90 weeks of permanent pasture.

At the beginning of each experiment the pasture was in very short, recently-grazed condition, the sward being dominantly ryegrass and white clover. At that time the sward of Experiment 1 covered 85 per cent of the ground surface, and was 55 per cent clover; that of Experiment 2 covered 75 per cent of the ground, and was 35 per cent clover. That of Experiment 3 covered 74 per cent of the ground, and was 42 per cent clover.

Fertiliser materials

Basal dressings included double superphosphate containing 45 per cent P_2O_5, gypsum containing 17 per cent sulphur, finely ground elemental sulphur, muriate of potash containing 60 per cent K_2O, and sodium molybdate. Nitrogen fertiliser used was calcium ammonium nitrate as "Nitrolime" (20.5 per cent N).

Methods of measurement and analysis

All harvests were made with a Gravely sickle-bar type mower cutting at approximately 2 in. above the ground surface with no return of clippings. For each plot the green weight and dry matter percentage were determined, and a sample dissected into species and dried to determine botanical composition of the harvest. The grasses and clovers of this last sample from Experiments 1 and 2 were retained after drying, for total nitrogen determination by the Kjeldahl method. No nitrogen determinations were made in Experiment 3.

Treatments

Experiment 1 comprised 10 manurial treatments, replicated four times in randomised blocks. All manures were broadcast on 26.9.57. A basal dressing of 200 lb per acre of double superphosphate, 200 lb of gypsum, 100 lb of muriate of potash, and 4 oz of sodium molybdate was applied in nine treatments, the tenth receiving no manure. Three of the plots receiving basal dressing were harvested after 48 days, three similar plots and the one receiving no manure were harvested for the first time after 105 days, and the final three plots were harvested for the first time after 154 days. Each of the above groups of three plots receiving basal dressings contained three Nitrolime treatments, representing 0, 50, and 100 lb N per acre. Plots measured 20 × 25 links, and harvested area was 0.002 acre.

Experiment 2 comprised the following five treatments replicated four times in randomised blocks: no nitrogen, 50 lb, 100 lb, 200 lb, 400 lb per acre of nitrogen applied as Nitrolime. All plots received a basal dressing similar to that used in Experiment 1. All manures were broadcast on plots of the same size as in Experi-

ment 1 on 16.11.57. Harvests on 0.002 acre per plot were made of all plots on 14.1.58, 6.3.58, and 1.5.58.

Experiment 3 was similar in character to Experiment 1, comprising three nitrogen treatments: N0, N100, N200 (each as Nitrolime) for each of four periods commencing 22.8.58 of 69 days, 77 days, 102 days, and 117 days. The basal dressing was identical with that used in Experiments 1 and 2 but for the addition of 20 lb per acre of sulphur. The 12 treatments were replicated four times in randomised blocks.

Meteorological conditions

Table 1 presents a record of climatic conditions kept by the Department of Agriculture at Broken River. The values of temperature are not strictly comparable to official records for other stations since Six's thermometers were used and records were taken only on days when visits to the experimental area were made for other purposes. The number of readings taken each month varied from about 12 in the winter to about 20 in summer.

TABLE 1. CLIMATIC CONDITIONS AT BROKEN RIVER
1.9.57—31.12.58

Month	Pptn (ins.)	Screen temperature °F			Soil 4 in. Mean °F
		Min.	Mean	Max.	
1957 Sep.	2.0*	n.r.	n.r.	n.r.	n.r.
Oct.	8.0*	n.r.	n.r.	n.r.	n.r.
Nov.	8.0*	n.r.	n.r.	n.r.	n.r.
Dec.	9.8	38.5	51.3	64.1	n.r.
1958 Jan.	4.9	44.7	58.0	71.4	n.r.
Feb.	3.7	47.4	59.4	71.5	n.r.
Mar.	3.0	43.2	56.3	69.4	n.r.
Apr.	4.3	32.2	45.9	59.7	44.2
May	5.2	32.8	43.9	55.0	39.8
June	1.4	26.7	39.1	51.6	36.4
July	1.5	21.3	34.5	47.8	31.4
Aug.	1.9	28.1	40.4	52.8	36.2
Sep.	1.5	25.5	42.3	59.2	39.1
Oct.	2.3	36.8	50.6	64.4	47.7
Nov.	1.5	37.9	53.0	68.1	53.3
Dec.	4.4	44.5	56.5	68.5	58.0

* Estimated from Castle Hill records (3 miles distant). n.r. = no record made.

The records illustrate the marked difference in spring moisture supply between 1957 and 1958. Calculations of potential evapotranspiration (B. Molloy pers. comm.) reveal serious moisture deficits in October and November 1958, and marginal deficits in March 1958. The slow rise of soil temperatures in the spring is similarly illustrated.

RESULTS

Experiment 1

Rate of spring herbage production. Results of the three serial harvests in terms of grasses, clovers, and total dry matter are illustrated in Fig. 1. Total dry matter increment rate on N100 plots can be interpreted from this figure as a value in excess of 150 lb per day during December. Highly significant responses to nitrogen were measured in the yield of grasses, principally ryegrass and tall oat grass, at all three harvests. In this wet season, however, serious losses of grass dry matter through respiration and decay in the standing herbage occurred after 105 days. More than 8,000 lb of dry matter were grown from N100 treatment in 105 days, and light became a serious limiting factor to further grass growth in such stands when left uncut.

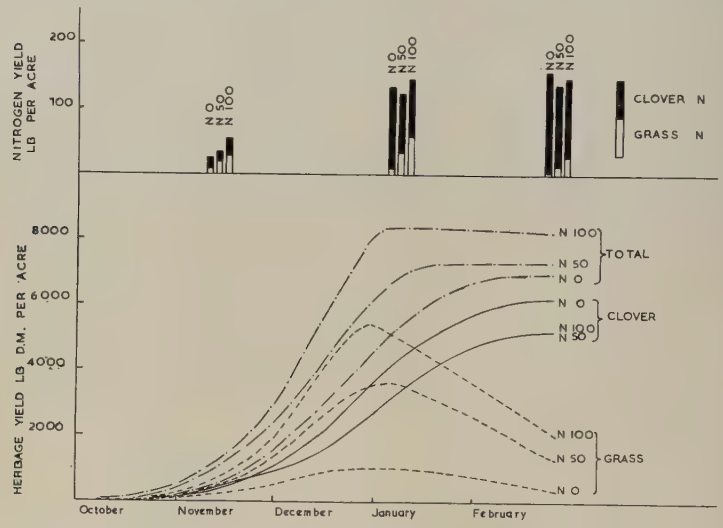

Fig. 1.—Herbage growth in uncut pasture at three levels of nitrogen during 1957–8 season, and yields of nitrogen in herbage at three stages.

Highly significant increases in clover yield, principally alsike and white clover, were measured at the first harvest in response to added nitrogen. At the second and third harvests a negative response to added nitrogen was measured in the clover yield. Dissection of samples indicated that this depression was in white

clover, in contrast with alsike clover which continued to show a response to nitrogen at levels up to 100 lb. Red clover remained a relatively minor constituent until the third harvest when it became co-dominant with alsike. At this last serial harvest the depression in clover yield from nitrogen-manured plots was relatively smaller than at the second harvest. No difference in clover yield depression between N50 and N100 treatments was measured at either the second or third harvests. The effect of the basal dressing on clover production was measured at the second harvest only. A 40 per cent increase in clover resulted from the extra fertiliser used in the basal dressing and was accompanied by a 25 per cent reduction in the yield of grass.

Nitrogen in herbage. Nitrogen analyses at the first harvest showed significant increases in nitrogen percentage of grasses with each increment of nitrogen applied but a depression which did not reach significance in nitrogen percentage of clovers with use of fertiliser nitrogen. At subsequent harvests no such treatment differences were detected. With the exception of the first harvest, therefore, total nitrogen uptake in grass and clover herbage, illustrated in Fig. 1, tended to follow closely the pattern of yields of dry matter. At the first cut significant increases in nitrogen harvested in grass, clover, and total herbage were attributable to nitrogen applications. At the second and third harvests nitrogen applications gave significant increases in yield of grass nitrogen and a significant depression in yield of clover nitrogen.

Experiment 2

Herbage production. Results of harvests are presented in Tables 2 and 3. Each letter indicates values not differing according to Duncan's multiple range test. Lower case and upper case letters are for the 5 per cent and 1 per cent significance level respectively.

TABLE 2. GRASS AND CLOVER YIELDS (SUMMER 1957–8)
(lb/ac dry matter)

Treatment	Harvest 14.1.58		Harvest 6.3.58	
	Grass	Clover	Grass	Clover
1. N0	1,430 d D	1,760 a AB	100 d D	1,930 a A
2. N50	2,490 c CD	2,220 a A	210 d CD	1,600 ab AB
3. N100	4,300 b B	1,550 ab AB	570 c C	1,100 bc BC
4. N200	6,020 a A	580 b B	1,710 b B	750 cd C
5. N400	6,880 a A	570 b B	3,380 a A	510 d C

The following are the main features of Table 2:

(i) The application of 50 lb nitrogen almost doubled the yield of grass in the 60 days of early summer without concurrent or subsequent depression in clover yield.

(ii) The application of 100 lb nitrogen gave larger and more lasting increases in grass yield together with a significant depression in clover yield in the late summer period.

(iii) High levels of nitrogen outstandingly increased grass yield and significantly depressed the yield of clover throughout the summer.

TABLE 3. TOTAL HERBAGE PRODUCTION (NOVEMBER TO APRIL)
(lb/ac dry matter)

Treatment	Individual harvests			Total yield
	14.1.58	6.3.58	1.5.58	
1. N0	3,230 d D	2,090 bc B	30 e E	5,350 d D
2. N50	4,750 c C	1,890 c B	40 d B	6,680 c CD
3. N100	5,910 b BC	1,830 c B	80 c C	7,820 c C
4. N200	6,730 ab AB	2,690 b B	120 b B	9,540 b B
5. N400	7,510 a A	4,080 a A	280 a A	11,870 a A

The following are the chief features of Table 3:

(i) Nitrogen applications in November all gave significant increases in total yield for the summer and autumn, increases in grass production more than compensating for any depressions in clover production.

(ii) Only nitrogen applications of 200 lb or more gave significant increases in total dry matter after the first harvest.

(iii) Summer pasture production in a wet season reached high levels, while autumn production was very low in spite of significant responses to nitrogen at higher levels of application.

The yields of nitrogen in grass and clover for the first and second harvests are presented in Table 4.

TABLE 4. NITROGEN RECOVERED IN HERBAGE GROWN
(16.11.57 TO 6.3.58)
(lb/ac N)

Treatment	Grass	Clover	Total
1. No	12.5 d D	116.5 a A	129.2 c B
2. N50	24.2 d D	110.5 a A	134.8 c B
3. N100	59.5 c C	72.0 c AB	131.5 c B
4. N200	132.8 b B	43.2 cd B	176.0 b B
5. N400	226.2 a A	31.8 d B	258.0 a A

It is clear from Table 4 that although treatments greatly affected the relative proportions of clover nitrogen and grass nitrogen, only applications of 200 lb or more of nitrogen significantly increased the total yield of nitrogen.

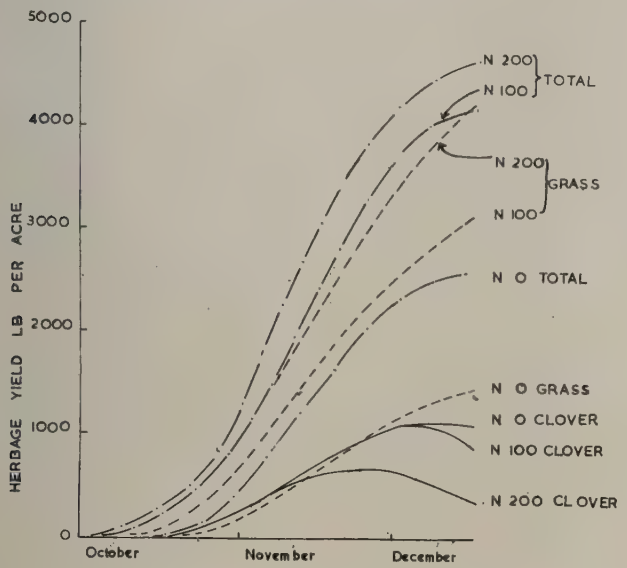

Fig. 2.—Herbage growth in uncut pasture at three levels of nitrogen during spring and early summer 1958.

Experiment 3

The results of four serial harvests up to full flowering of ryegrass are illustrated in Fig. 2. Although manures were applied in August 1958, no measurable growth occurred until early October. Maximum daily rate of dry matter increment is indicated to be approximately 100 lb during November 1958. The increase in clover at the first harvest did not reach significance at the 5 per cent level. The second harvest showed no significant changes in clover yield with increments of nitrogen, but a significant depression with N200 was recorded by the third harvest. Significant responses to nitrogen were measured in grass and total yield through all harvests.

DISCUSSION

Influence of nitrogen fertiliser on herbage production

From the experience of two seasons, the first much wetter than normal, the second drier than normal, an appreciation has been gained of the role of nitrogen in raising the rates of herbage production closer to the ceiling determined both by climate and by

the genetic capability of the plants used. In both seasons the early spring rate of pasture growth was doubled and the maximum rate of total dry matter increment was increased by at least 50 per cent by the application of 100 lb per acre of nitrogen. This last effect occurred during the late spring and early summer period corresponding to a phase of high growth potential associated with culm elongation of the dominant grass species as outlined by Campbell (1961). The earlier phase is characterised by high nitrogen uptake without proportional increase in dry matter, the later phase by the fulness of reproductive development.

From the 110 day yield of the first two periods of Experiment 2 functions have been calculated for total herbage and grass herbage. The regression for total herbage yield (Y) on fertiliser nitrogen (F_n)

$$Y = 15.20F_n + 5862.8$$

is highly significant, and there is no evidence of significant departure from this linear form.

In the case of grass herbage yield (G), the linear regression

$$G = 21.86F_n + 2175.2$$

is highly significant but does not completely represent the real relation. Calculation of a quadratic function resulted in an improvement which reached significance at the 10 per cent level but still did not explain fully all the variation in G. It appears as though a logistic curve would best fit the data but insufficient points are available for testing it.

Competition between clovers and grasses

The positive response to nitrogen fertilisers has been shown in both clovers as well as grasses, and white and alsike clovers have exhibited a positive growth response to added nitrogen in spring, and only very tall and heavy growth of competing grass has been associated with reduction in clover. Under the prevailing weather of the 1957 8 season with long intervals between mowings the suppression of clover by vigorous nitrogen-promoted grass growth was paralleled by the suppression of grass on no-nitrogen plots that received the legume-promoting basal dressing of sulphur, phosphorus, molybdenum, and potash. This indicates that under the soil nitrogen conditions prevailing at this early phase of development in the region, a high grass to clover ratio can be maintained in herbage harvests only by frequent defoliation or by withholding manures necessary for optimum legume growth. This situation is partially in contrast with the more usual conditions of better nitrogen supply where infrequent defoliation allows clover suppression.

Factors affecting yield of nitrogen in grass

A general proposal for yield of grass nitrogen considered as a function of clover nitrogen and fertiliser nitrogen has been made by

Walker *et al.* (1954). If the data of Experiment 2 are used to plot the function $G_n = K_1(C_n + F_n) + K_2$ as proposed by Walker *et al.* (*loc. cit.*) an approximately linear expression can be obtained in which K_1 and K_2 have values similar to those obtained in the Brilley experiment, the most clovery sward (op. cit.). This parallel apparent relation of G_n to C_n and F_n is not sustained when correlation coefficients and multiple regression coefficients are calculated from the Broken River data in the form of the equation $G_n = bC_n + cF_n + K$ that is also proposed by Walker *et al.* (*loc. cit.*). Comparable values for the present Experiment 2 and that at Lincoln described by Walker *et al.* (1953) are set out below. The values for the Lincoln experiment have been recalculated from the original data kindly furnished by one of the authors (Adams A.F.R. pers. comm.) in the same manner as the Broken River values.

	Broken River	*Lincoln*
G_n, C_n	-0.932^{**}	-0.638^{*}
G_n, F_n	$+0.994^{***}$	$+0.965^{**}$
C_n, F_n	-0.909^{**}	-0.776^{**}
b	-0.380 ± 0.330	$+0.661 \pm 0.256$
c	$+0.474 \pm 0.080$	$+0.692 \pm 0.063$

In the case of Broken River experiment b is not significant, and the inclusion of C_n does not add to the accuracy of the equation. The simple linear regression

$$G_n = 0.558F_n + 7.29 \pm 0.035$$

is highly significant and explains all the variation in G_n apart from a small but significant remainder. A sigmoid curve for F_n

$$G_n = 118 + 118 \tanh (0.007348F_n - 1.33173)$$

explains all variation in G_n but lacks sufficient points to obtain significance at less than 10 per cent.

Since the relation of G_n to F_n is apparently sigmoid, owing to competition for applied nitrogen between grasses and clovers at the 50 lb N level and the incomplete utilisation of applied nitrogen at the 400 lb N level, it is not expected that a relation would appear between the three variables G_n C_n, F_n identical in form and similar in values to that derived from the Lincoln experiment. However, it is clear from the Broken River experiment that long intervals between defoliations foster competition for light between grasses and legumes, and therefore promote an inverse relation between grass nitrogen and clover nitrogen. It has been shown by Butler *et al.* (1959) that frequent defoliations of white clover promote underground transfer of nitrogen from root and nodule shedding. Therefore it is likely that the relation derived by Walker and his associates by multiple regression analysis from the Lincoln expiriment may be more apparent than real, since different frequencies of defoliation were practised in that experiment in order to obtain different proportions of clover. Recalculations of the Lincoln data using mowing frequency (M) as a variable in addition to C_n and F_n show that the best fit is obtained by the regression in which C_n is omitted:

$$G_n = 0.453F_n + 14.322M + 77.26 \pm 0.046 \quad \pm 3.974.$$

The conclusion is drawn that in the short term, as represented by the Broken River experiment, grass nitrogen yield from a mixed grass-legume sward under different levels of fertiliser nitrogen may respond linearly over a large range of applied nitrogen levels. Clover may so little affect the primary relation of grass nitrogen to fertiliser nitrogen that grass nitrogen yield in a mixed sward may simulate that from a non-leguminous sward as described by Walker (1956). Long-term experiments in which mowing frequencies are carefully studied are necessary in order to interpret the competitive or additive roles of fertiliser nitrogen and clover nitrogen in affecting the yield of grass nitrogen.

Practical conclusions

This work demonstrates the high potential for grassland production in this environment in the short season of active growth. It also demonstrates the need for high levels of available nitrogen to achieve this potential of grass production. If for reasons of economy this increase in available nitrogen is to be gained from bacterial fixation rather than from fertiliser nitrogen, then it is clear that continued emphasis must be placed in grassland management on the promotion of legume growth even at the expense of the associated grasses. This condition of deliberately maintained legume dominance may be necessary for several years before soil nitrogen supplies are built up to a level close to that required for maximum grass growth. It is therefore unlikely that primary cultivation for establishment of pastures containing good grasses is economically desirable as a programme of land development in such regions if legume establishment and legume dominance can be secured by cheaper oversowing and top-dressing methods.

This work also demonstrates that the largest absolute gain in yield of the sown grasses attributable to high nitrogen supply is to be obtained in late spring and early summer rather than in the cooler periods of greater livestock feed deficiency. While relative gains in herbage production are high in early spring and autumn the low absolute yields of grass in these periods indicates that research effort should be directed at testing new grass strains for cool season production and that the practical difficulties of distributing feed supplies to meet animal needs must be overcome for the present by herbage conservation practices such as hay and silage making and the saving of foggage and of summer grown pasture.

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REFERENCES

Butler, G. W.; Greenwood, R. M.; Soper, K. 1959: Effects of shading and defoliation on the turnover of root and nodule tissue of plants *Trifolium repens*, *Trifolium pratense* and *Lotus uliginosus*. *N.Z. J. agric. Res. 2:* 415–26.

Campbell, A. G. 1961: A theoretical basis for grazing management. *Proc. N.Z. Soc. Anim. Prod. 21:* 18–32.

Dingwall, A. R. 1956: Tussock grassland research in the Broken River area. *N.Z. J. Agric. 93:* 277–83.

O'Connor, K. F. 1959: Plant production in difficult environments. *Proc. N.Z. Inst. Agric. Sci. 5:* 86–94.

Walker, T. W. 1956: Nitrogen and herbage production.. *Proc. 7th int. Grassl. Congr.* 157–65.

Walker, T. W.; Adams, A. F. R.; Orchiston, H. D. 1953: The use of fertilizers on herbage cut for conservation Pt. IV. The effects of rates, method of application and forms of fertilizer nitrogen on the yields of dry matter and nitrogen of grasses and clovers in a ryegrass and white clover sward, cut at different stages of growth. *J. Brit. Grassl. Soc. 8:* 281–99.

Walker, T. W.; Orchiston, H. D.; Adams, A. F. R. 1954: The nitrogen economy of grass legume associations. *Ibid. 9:* 249–74.

NITROGEN AND GRASSLAND PRODUCTION IN THE MID-ALTITUDE ZONE OF CANTERBURY, NEW ZEALAND

II. The effects of different levels of calcium-ammonium nitrate on yields of cultivated pastures under different mowing frequencies

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(Received 7 March 1961)

ABSTRACT

Experiments are reported showing the influence of mowing patterns and of nitrogenous fertiliser on mixed grass-clover pastures sown after cultivation. Mowing times or mowing frequency affected differentially the principal components of pasture, especially in altering relative competitive ability for light under luxuriant growing conditions. Nitrogenous fertiliser had a larger effect on grass yield under infrequent late spring-early summer defoliation, attributable in part to increased ability of grass to compete with clovers for light in such conditions and in part to the non-interruption of growth of the main crop of grass tillers.

In contrast with many lowland conditions with long growing seasons, grass yield is shown to be a direct function of fertiliser nitrogen and a reciprocal function of number of mowings. The results are interpreted in terms of principles of pasture production developed from experience in other environments. Some practical implications for grassland use in this environment are briefly discussed.

INTRODUCTION

The effects of varying levels of nitrogen fertiliser on the clovery pastures of the Broken River Experimental Area, at 2,350 ft above sea level, in the Waimakariri Catchment, have been reported by O'Connor (1961). It was shown that for the warmer part of the growing season significant increases in yield of both grass and total dry matter and of grass nitrogen were obtained with increasing applications of calcium-ammonium nitrate as "Nitrolime" up to 400 lb N per acre. The yield of both clover dry matter and clover nitrogen declined with increments of fertiliser nitrogen above 50 lb N per acre. It was also shown (O'Connor

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1959) that the use of "Nitrolime" had greatly raised the rate of dry matter increment in pastures uncut from the commencement of spring growth to the full flowering of ryegrass.

Two experiments described in the first paper of this series as Number 1 and Number 3 were continued to test the effects of different mowing frequency patterns. The present paper reports the results of these trials during 1957–8, 1958–9, and 1959–60.

EXPERIMENTAL

Pastures, manurial treatments, and methods of measurement and analyses of herbage have been described in the first paper of the series.

Treatments. Dates of harvesting of plots of Experiment 1 are set out as follows for 1957–8, all N levels being lb of nitrogen per acre as "Nitrolime":

N0, N50, N100 with basal dressing: 13.11.57, 27.2.58 (referred to as "early and late").

N0, N50, N100 with basal dressing, and "no manure" (no basal dressing): 9.1.58, 27.2.58 (referred to as "mid and late").

N0, N50, N100 with basal dressing: 27.2.58 (referred to as "late only").

Dates of harvesting of Experiment 2 (referred to as Experiment 3 in first paper of series) are set out below for the 1958–9 season. Three "Nitrolime" levels were included at each mowing frequency: N0, N100, and N200, measured in lb of nitrogen per acre.

Mowing Frequency	*Dates*
Six cuts:	30.10.58, 2.12.58, 8.1.59, 6.3.59, 6.4.59, 24.4.59.
Four cuts:	7.11.58, 17.12.58, 6.3.59, 24.4.59.
Three cuts:	2.12.58, 6.3.59, 24.4.59.
Two cuts:	17.12.58, 24.4.59.

Basal dressings of sulphur, phosphorus, and potash, together with "Nitrolime" as before, were reapplied on 15.9.59. During the 1959–60 season plots were harvested according to the following schedule:

Six cuts:	12.11.59, 1.12.59, 22.12.59, 29.2.60, 10.3.60, 21.3.60.
Four cuts:	19.11.59, 22.12.59, 29.2.60, 21.3.60.
Three cuts:	1.12.59, 20.1.60, 21.3.60.
Two cuts:	22.12.59, 21.3.60.

During 1959 and 1960 harvests were made with a Morrison reel-type mower, instead of the Gravely sickle-bar type mower used earlier in these experiments. This modification gave more effective collection of clippings during drought conditions, the sward being clipped approximately 0.5 in. to 1 in. shorter than described earlier for the sickle-bar mower. Harvested area was 0.002 acres per plot in Experiment 1, and 0.0012 acres per plot in Experiment 2.

TABLE 1. YIELDS OF HERBAGE AND NITROGEN 26.9.57–27.2.58

Treatment		Herbage lb/ac of dry matter			Nitrogen lb N/ac		
Cutting	Manure	Grass	Clover	Total	Grass	Clover	Total
Early	SPKMo N0	760 ef DE	4,610	5,890 g	12 d EF	106	118
and	„ N50	1,460 d CD	4,340	6,470 fg	27 c CD	111	138
Late	„ N100	2,090 c C	4,770	7,770 cde	41 b B	116	157
Mid	„ N0	1,270 de D	6,510	7,920 cd	14 d EF	204	218
and	„ N50	3,890 b B	5,600	10,060 b	42 b B	180	222
Late	„ N100	5,420 a A	5,510	11,170 a	63 a A	171	234
Late	„ N0	450 f E	6,230	6,990 def	4 e F	153	157
Only	„ N50	1,390 d CD	5,220	7,300 cdef	14 d EF	123	137
	„ N100	2,160 c C	5,330	8,210 c	27 c C	119	146
Mid and Late	Nil	1,580 cd CD	4,840	6,700 efg	17 d DE	154	170
Means							
Levels of Nitrogen (N)							
(a) N0		830 b	5,780 a A	6,930 b A	10 c B	154 a	164 a
(b) N50		2,250 ab	5,050 b B	7,940 ab A	28 b AB	138 b	166 a
(c) N100		3,220 a	5,200 b B	9,050 a A	44 a A	135 b	179 a
Cutting Times (C)							
(a) Early and Late		1,440 b	4,570 b B	6,710 b B	27 ab A	111 c B	138 b B
(b) Mid and Late		3,530 a	5,870 a A	9,720 a A	40 a A	185 a A	225 a A
(c) Late only		1,330 b	5,590 a A	7,500 b AB	15 b A	132 b B	147 b B
Interaction							
N × C		**	N.S.	*	**	N.S.	N.S.

Meteorological conditions. A climatic summary for the Broken River Experimental area for the 1957 and 1958 seasons has been presented in the first paper of this series. The seasons 1958–9 and 1959–60 were both droughty, especially 1958–9, when spring rainfall was considerably below average in the district. Precipitation recorded is shown as part of Figs. 1, 2, and 3.

RESULTS

Experiment 1. Yields of herbage and of herbage nitrogen recovered during the period 26.9.57 to 27.2.58 are summarised in Table 1.

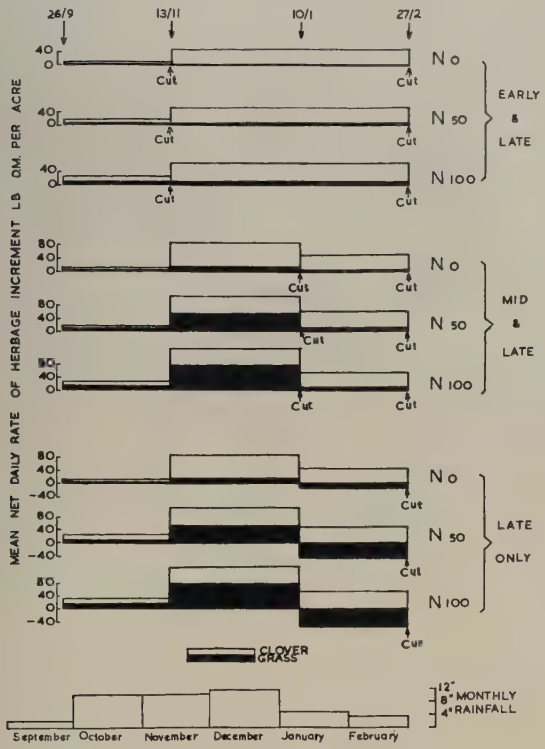

Fig. 1.—Pasture growth rates in 1957–8 as affected by time of mowing and level of applied nitrogen.

The highly significant interaction between nitrogen levels and cutting treatments in grass yields indicates the importance of cutting treatments in controlling the expression of the response of grass to fertiliser nitrogen. The fullest response to nitrogen fertiliser was shown only in the "mid and late" series. Yields from

this series were consistently superior to those from plots not cut in the mid-summer period.

Daily rates of dry matter increment under the different treatments are illustrated in Fig. 1, as means for the three periods: 26.9.57–13.11.57, 13.11.57–9.1.58, 9.1.58–27.2.58. The assumption of homogeneity within blocks is made in calculating these rates for second and third periods. The net negative rate of dry matter increment in grass uncut until 27.2.58, illustrated for the third period, is attributable to the decay occurring under the heavy clovery canopy.

Very little growth occurred after the harvest of 27.2.58. All plots were harvested again on 7.5.58, and, although there were no

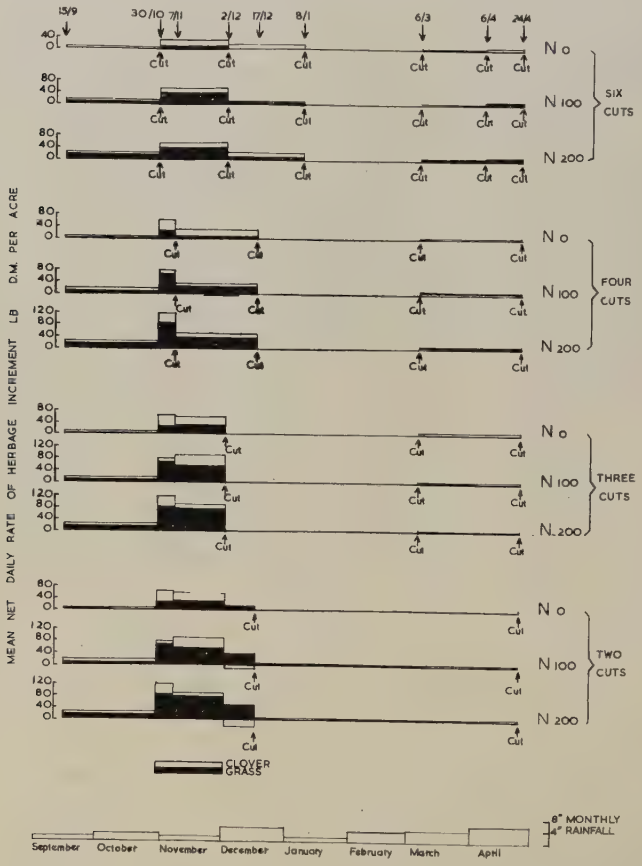

Fig. 2.—Pasture growth rates in 1958–9 as affected by frequency of mowing and level of applied nitrogen.

further responses to applied nitrogen, the autumn yield of plots mown "mid and late" was only half (70 lb/ac dry matter) that from the "early and late" plots and the "late only" plots.

Experiment 2. Yields of herbage during 1958–9 season are presented in Table 2.

TABLE 2. YIELDS OF HERBAGE 1958–9 SEASON
(lb/ac dry matter)

Treatment		Grass	Clover	Total
Cutting	Manure			
6 times	SPKMo N0	780	1,600	2,340
"	" N100	2,190	1,040	3,390
"	" N200	2,850	980	4,060
4 times	SPKMo N0	860	1,420	2,320
"	" N100	2,310	930	3,220
"	" N200	3,340	980	4,370
3 times	SPKMo N0	1,340	1,250	2,700
"	" N100	2,850	1,210	4,240
"	" N200	3,840	740	4,750
2 times	SPKMo N0	1,600	1,270	2,960
"	" N100	3,460	1,110	4,780
"	" N200	4,610	540	5,230
Means				
Rates of N	N0	1,150 C	1,390 A	2,580 C
	N100	2,700 B	1,070 B	3,900 B
	N200	3,660 A	810 C	4,600 A
Cutting times	6	1,940 c C	1,210 a A	3,260 c B
	4	2,170 c C	1,110 ab A	3,330 c B
	3	2,680 b B	1,070 ab A	3,900 b A
	2	3,230 a A	970 b A	4,320 a A
Interactions				
N × C		n.s.	n.s.	n.s.

Principal features of Table 2 are: (i) the clear-cut effect on yield of grasses of cutting frequency as well as of level of fertiliser nitrogen; (ii) the lack of highly significant difference in clover yield from different cutting treatments.

Yields of herbage during 1959–60 after re-application of both cutting and manurial treatments are presented in Table 3. Effects similar to those of the previous season are shown, but the effect of cutting frequency on clover yield appears even smaller than in 1958–9.

The dominating influence of the late spring-early summer production period in both seasons is demonstrated in Figs. 2 and

TABLE 3. YIELDS OF HERBAGE 1959–60 SEASON
(lb/ac dry matter)

Treatment		Grass	Clover	Total
Cutting	Manure			
6 times	SPKMo N0	1,210	1,730	2,930 g
„	„ N100	2,390	990	3,380 fg
„	„ N200	3,420	630	4,050 ef
4 times	SPKMo N0	1,670	1,860	3,430 fg
„	„ N100	2,660	1,270	3,920 ef
„	„ N200	3,610	820	4,420 de
3 times	SPKMo N0	2,140	1,830	3,970 ef
„	„ N100	3,740	1,100	4,840 cd
„	„ N200	4,570	830	5,400 bc
2 times	SPKMo N0	2,660	1,750	4,410 de
„	„ N100	4,700	1,130	5,830 b
„	„ N200	6,110	690	6,800 a
Means				
Rates of N (N)	N0	2,170 C	1,790 A	3,710 c B
	N100	3,370 B	1,120 B	4,490 b AB
	N200	4,430 A	740 C	5,170 a A
Cutting times (C)	6	2,340 c C	1,120 a	3,450 c C
	4	2,650 c C	1,320 a	3,960 c BC
	3	3,480 b B	1,250 a	4,740 b AB
	2	4,490 a A	1,190 a	5,680 a A
Interactions				
N × C		n.s.	n.s.	*

3, presenting pasture growth rates for the different treatments. It is clear from these figures that not only has production in this period dominated the total yield for each season but frequent defoliations have adversely affected pasture growth rate most severely at this time.

DISCUSSION

Walker *et al.* (1953) showed that increased frequency of mowing had tended to increase the yield of grass and also the yield of grass nitrogen at Lincoln in the Canterbury lowlands. In such environments, climate allows a relatively long production season and relatively frequent defoliation is required to maintain the clover component of swards. This may promote the shedding of clover roots and nodules and faster renewal of vigorous grass tillers. For these reasons it is not uncommon for excessive spelling between defoliations to result, as in the Lincoln experiment, in lower annual yields than those obtained from pastures defoliated somewhat more frequently. However, as Brougham (1957a) has pointed out in his

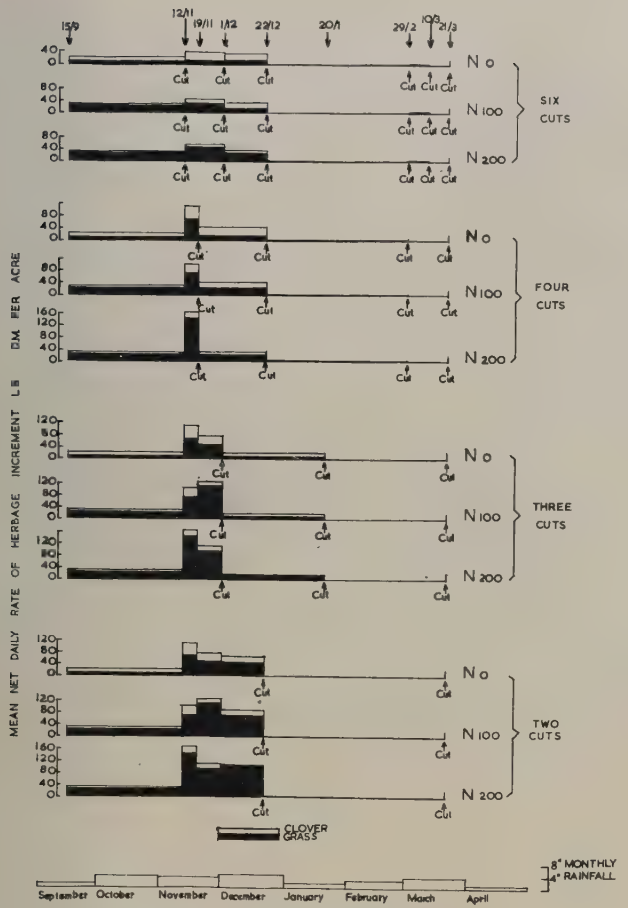

Fig. 3.—Pasture growth rates in 1959–60 (second year of treatment) as affected by frequency of mowing and level of applied nitrogen.

review of this subject, lengthened spells between defoliations have resulted generally in increased yields of pastures.

Mitchell (1956) has discussed the significance of tiller size and density to the annual production of pastures, from experience in climates where growth is possible over many months of the year. In conditions allowing much shorter growing periods, however, promoting tiller replacement may not be of such great significance to total annual production as permitting tillers already present to express their growth towards the maximum. Defoliation, while promoting tillering in such strains as the ryegrasses used in these experiments, also has the effect of interrupting the rate of growth

of tillers already present, as shown by Sullivan and Sprague (1943). If, as Langer (1959) suggests, very young tillers are slow in growth during their establishment, it is likely that changes in the age composition of the tiller population induced by defoliation could have appreciable effects on the rate of dry matter increment of the pastures. The effect of these phenomena is reinforced in pasture conditions by the incomplete utilisation of light which occurs until closely defoliated pastures have recovered to an effective leaf area. Brougham (1955) has demonstrated this effectively, establishing that the rate of regrowth of pasture following close defoliation describes a sigmoidal curve with respect to time. Such a situation has also been described by O'Connor (1956) in discussing experiments with Kentucky bluegrass/cocksfoot/white clover pastures in central New York State, an environment somewhat similar in the seasonal rhythm of pasture growth to that at Broken River. The sigmoid character of the curve of dry matter increment at Broken River over several weeks from the beginning of spring has been shown by O'Connor (1961) from the results of both a wet and a droughty season. These curves are all closely similar to that described for spring growth at Palmerston North by Brougham (1957b). It is believed from observations, although no measurements of yield have been made at stages during growth after defoliations later in the season, that the sigmoid character of pasture regrowth has been repeated after successive defoliations during these experiments at Broken River.

Results of the first year of Experiment 1 show clearly that the design of treatments was unsuited to the production level experienced in that wet season. All three cutting series suffered from conditions of interplant competition for light, described as Phase III growth by Brougham (*loc. cit.*), at one or more stages during the growing season. Nitrogen top-dressings not merely affected the production of the grasses directly but also influenced the ability of the grasses to compete with clovers for limited light. The cumulative effect of these influences of nitrogen on grass yield is shown in the three- or four-fold increases with increments of 50 or 100 lb N under cutting treatments which allowed grass to grow without interruption through the period of peak production in late spring–early summer. Defoliation in November induced a further period of Phase I growth, and thereby reduced the yield of both grasses and clovers, and greatly depressed the level of response in grass to applied nitrogen. Failure to defoliate at all until the end of February caused prolonged Phase III growth, and resulted in net loss of grass dry matter by decomposition, most serious on N100 plots as shown in Fig. 1.

In the two year course of Experiment 2 weather conditions at no time allowed the same degree of bulky clover growth as was experienced in 1957–8. However, it is apparent that Phase III growth occurred in December, especially in 1958, and that clover yields in particular suffered accordingly, the depression in clover being more serious on plots with added nitrogen.

The significance of both level of applied nitrogen and frequency of cutting to grass production is shown in the regressions for grass yield on number of mowings and level of nitrogen presented below. G represents grass yield in lb of dry matter per acre, M is number of mowings per season, and F_n is level of fertiliser nitrogen applied.

$$\text{1958–9 Season:} \quad G = 12.59F_n + \frac{3983}{M} + 1 \pm 0.78 \pm 487$$

$$\text{1959–60 Season:} \quad G = 12.52F_n + \frac{6717}{M} - 113 \pm 0.89 \pm 587.$$

Because of the dominating contribution of late spring–early summer production to total annual production in the 1958 9 and 1959–60 seasons at Broken River, the effect of increased frequency of cutting has been principally deleterious, through the repeated induction of Phase I growth. The beneficial effect that greater frequency of cutting might have had on tiller populations of both grasses and clovers has not been expressed in yield under the climatic and soil conditions existing during summer and autumn. This feature may in large measure account for the much greater relative difference in annual total yields attributable to mowing frequency in the Broken River experiment than that measured by Brougham (1957b) at Palmerston North in his comparison of frequent and infrequent hard grazings. A similar explanation, with more emphasis on nitrogen economy, may be offered for the reversal of the situation recorded by Walker *et al.* (1953) at Lincoln.

Weather conditions immediately following different defoliations may have differentially affected regrowth after the manner proposed by Jantii and Kramer (1956), but any such effects have apparently not gravely modified the overall influence of nitrogen supply and mowing frequency.

Practical conclusion. If maximum yield is to be obtained from the grass component of cultivated pastures frequent defoliation must be avoided during the late spring-early summer period. Such a practice runs counter to the objective of promoting clover growth for soil fertility building, more especially in dry years.

The relative failure of the dominantly white clover legume component of pasture to profit from low nitrogen or frequent defoliation conditions during the two dry years of Experiment 2 points, on the one hand, to the need for pasture management to maximise every opportunity for legume growth and nitrogen fixation in the current land improvement phase, and indicates, on the other hand, the possible usefulness of more drought-tolerant legumes such as lucerne.

The joint effect of nitrogen application and infrequent defoliation in promoting growth during late November and December offers considerable promise for silage and hay production when

soil fertility conditions are improved by management of clovery swards.

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REFERENCES

Brougham, R. W. 1955: A study in rate of pasture growth. *Aust. J. agric. Res. 6:* 804–12.

————1957a: Some factors that influence the rate of growth of pasture. *Proc. 19th Conf. N.Z. Grassl. Ass.* pp. 109–16.

————1957b: Pasture growth rate studies in relation to grazing management *Proc. N.Z. Soc. Anim. Prod. 17:* 46–55.

Jantii, A.; Kramer, P. J. 1956: Regrowth of pastures in relation to soil moisture and defoliation. *Proc. 7th int. Grassl. Congr.* pp. 33–44.

Langer, R. H. M. 1959: Growth patterns of grasses in the sward. *Proc. N.Z. Inst. agric. Sci. 5:* 53–9.

Mitchell, K. J. 1956: The influence of light and temperature on the growth of pasture species. *Proc. 7th int. Grassl. Congr.* pp. 58–69.

O'Connor, K. F. 1956: "Influences of treading on pastures." p. 90 Ph.D. thesis, Cornell Univ., Ithaca, N.Y. University Microfilms, Ann Arbor, Michigan. 156 pp.

————1959: Plant production in difficult environments. *Proc. N.Z. Inst. agric. Sci. 5:* 86–94.

————1961: Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. I. *N.Z. J. agric. Res. 4:* 686–97.

Sullivan, J T.; Sprague, V. G. 1943: Composition of the roots and stubble of perennial ryegrass following partial defoliation. *Plant Physiol. 18:* 656–70.

Walker, T. W.; Adams, A. F. R.; Orchiston, H. D. 1953: The use of fertilizers on herbage cut for conservation. IV. *J. Brit. Grassl. Soc. 8:* 281–99.

NITROGEN AND GRASSLAND PRODUCTION IN THE MID-ALTITUDE ZONE OF CANTERBURY, NEW ZEALAND

III. The effects of nitrogenous and other fertiliser materials on uncultivated pastures

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(Received 7 March 1961)

ABSTRACT

An account is given of three experiments conducted on uncultivated grasslands in the fescue tussock zone of the Broken River Basin. Where other nutrients were not limiting, constant returns in grass yield were shown to increments of nitrogen up to 200 lb N per acre. On both poor and good sites grass yield was increased fivefold with applications of nitrogen at the level of 200 lb N per acre. Increases were shown to persist for at least two years after fertiliser application on the poor sites.

In the presence of adequate supply of other nutrients the adventive grasses, especially Yorkshire fog, tended to be superior to native grasses in response to nitrogen applications. Where sulphur and phosphate were in scarce supply, browntop responded more to fertiliser nitrogen than did sweet vernal.

In discussion of results some changes are predicted in pasture production and composition as the outcome of different manuring and grazing management policies.

INTRODUCTION

Two earlier papers in this series (O'Connor 1961a, 1961b) have given an account of experiments with nitrogenous fertilisers on cultivated pastures at the Broken River Experimental Area of the Department of Agriculture, at 2,350 ft above sea level in the Waimakariri Catchment. In the mid-altitude zone of Canterbury topography is a barrier to the large-scale development of cultivated pastures, although such pastures are potentially of great significance because of their usefulness for the conservation of herbage. Considerable importance is attached to the hillside pastures of much wider extent and to their potential productivity with development. The spectacular success of

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The experiments here reported were all commenced while the author was Liaison Officer, Tussock Grasslands Research, Department of Agriculture, Christchurch. Since October 1959 they have been carried on as part of the research programme of Grasslands Division, D.S.I.R..

clover introduction into such uncultivated pastures by surface sowing with appropriate manuring techniques has been described by various authors (Walker *et al.* 1955, 1956; Lobb and Bennetts 1958; Lobb 1958; and O'Connor 1959). The levels of clover production which have been recorded from such development (O'Connor 1959; Walker *et al.* 1956) indicate the considerable improvement in the nitrogen supply to the resident grasses of uncultivated pastures which may ensue from development under appropriate grazing management. To obtain evidence on the likely effects of such improvement in nitrogen supply, the present experiments were commenced in 1957 and 1958, measuring herbage growth, production, and composition after the application of nitrogenous fertilisers at different levels and with different supplementary fertilisers. The only previously published account of the application of nitrogenous fertilisers to uncultivated pastures in the tussock grassland areas of South Island, New Zealand, is that of Walker and Adams (1958). In the work there discussed only relatively low rates of nitrogen were applied in the form of "Nitrolime" at a somewhat lower altitude.

EXPERIMENTAL

Three experiments are reported in the present paper. The first was located on a calcareous and loessial soil related to the Castle Hill soil at a site 3,000 ft above sea level, referred to as Hogsback. The second was located on Craigieburn soil, previously referred to as Cass soil, at the Broken River Experimental Area at 2,350 ft. The third was on a similar site at approximately 2,400 ft, referred to as Enys Flat. All three sites were situated in the Broken River Basin of the Waimakariri Catchment within four miles of one another. The Hogsback site, by reason of its proximity to the Craigieburn Mountains, enjoyed higher total precipitation than the other two sites. The soil there was deeper, more moist, and higher in carbon, nitrogen, and phosphate than the Craigieburn soil, as shown in Table 1. Whereas sulphur was the principal non-nitrogenous nutrient deficiency on the Hogsback site, phosphate and molybdenum were also deficient on the Craigieburn soil (Dingwall 1956, O'Connor 1959).

Pastures

Experiment 1 was on good low tussock grassland, the principal species of which were *Festuca novae-zelandiae* (Hack.) Cockayne, *Anthoxanthum odoratum* L., *Holcus lanatus* L., and *Agrostis tenuis* Sibth. Subsidiary in their contribution to the total cover and production were the following: *Danthonia unarede* Raoul, *Poa pratensis* L., *Poa caespitosa* Sprengel., *Agropyron scabrum* (R. Br.) Beauv., *Poa colensoi* Hook. f., *Festuca rubra* L., *Hieracium praealtum* Gochnat var. *decipiens* Koch., *Hypochaeris radicata* L., *Wahlenbergia albomarginata* Hook., *Cyathodes fraseri* (A. Cunn.) Allan, *Senecio bellidioides* Hook. f., *Acaena* spp., *Craspedia* sp., and *Trifolium repens* L. Red tussock *Danthonia* sp.) and several other small native herbs were present but were negligible in their contribution to total cover and production of

TABLE 1. CHEMICAL CHARACTERISTICS OF SOILS AT THREE EXPERIMENTAL SITES, BROKEN RIVER BASIN, CANTERBURY

Site	Depth (in.)	pH	Quick tests*			Total carbon %	Nitrogen	C/N
			Ca	K	P			
Hogsback	0–3	6.1	14	5	5	4.71	0.46	10
	3–6	5.8	11	2	4	2.81	0.26	11
Broken River	0–3	5.5	3	15	2	3.54	0.26	14
	3–6	5.0	1	4	1	2.63	0.20	13
Enys Flat	0–3	5.5	3	9	1	3.46	0.26	13
	3–6	5.1	1	3	1	2.71	0.21	13

* The indices used in quick tests are as follows:

Ca: parts per 40,000 of extract, using soil solution ratio of 1:5 (extractant solution: normal ammonium acetate).

K: parts per 250,000 of extract, using soil solution ratio of 1:5 (extractant solution as above).

P: parts per 50,000,000 of extract, using soil solution ratio of 1:200 (extractant solution: 0.002 normal Truog reagent buffered with ammonium sulphate to pH 3).

herbage. Total annual herbage production in undeveloped condition was estimated at 1,000 to 1,500 lb DM per acre, and cover 100 per cent.

Experiments 2 and 3 were on poor low tussock grassland, the principal species of which were *Agrostis tenuuis, Anthoxanthum odoratum, Festuca novae-zelandiae, Poa colensoi, Cyathodes fraseri, Hypochaeris radicata, Rumex acetosella* L., *Hieracium praealtum* var. *decipiens, Raoulia subsericea* Hook. f., *Rhacomitrium lanuginosum* (Hedw.) Brid. var. *pruinosum,* Hook. f. et Wils., and *Pimelea prostrata* (J. R. et G. Forst.) Willd. *sensu lato.* A few old plants of *Discaria toumatu* Raoul were eradicated from the site of Experiment 3 before treatments were applied. Total annual herbage production from the Broken River site was estimated at 150 lb DM per acre, and cover about 40 per cent. The site at Enys Flat was estimated to yield annually before development about 250 lb DM per acre, with cover about 60 per cent. In the Broken River site browntop and sweet vernal were the principal components of yield. In the Enys Flat pasture *Festuca novae-zelandiae* was a major component of yield, with browntop and sweet vernal of similar importance.

Fertiliser materials

The following materials were used as described in treatments: Double superphosphate containing 45 per cent P_2O_5, superphosphate containing 22 per cent P_2O_5, muriate of potash containing 60 per cent K_2O, gypsum containing 16.8 per cent sulphur, sulphur as finely ground sulphur, sodium molybdate, calcium ammonium nitrate as "Nitrolime" containing 20.5 per cent nitrogen, urea containing 46 per cent nitrogen.

Methods of measurement and analysis

Harvests of Experiment 1 were made from 6 sq. ft in all plots in 1957–8, from 0.001 acre per plot in 1958–9, and from 12 sq. ft per plot in 1959–60. Experiment 2 was harvested from 0.002 acre per plot in 1958–9, and from 6 sq. ft per plot in 1959–60. Experiment 3 was harvested from 0.002 acre per plot in 1958–9 and 1959–60. Experiments 1 and 2 were mown and cleared before fertiliser treatments were applied at the commencement of the trials. Experiment 3 was not pre-trimmed, and, although dead matter was dissected from all samples and discarded from the first harvest, it is possible that some living fescue tussock had grown in the pre-treatment phase. All plots of all experiments were separately sampled for dry matter and botanical composition after green weighing, herbage being cut and removed after each harvest. Point analysis was made with 200 points at paced intervals on all plots of Experiment 1 after they had been mown in the autumn of 1958. Only hits on vegetation within 1 in. of the ground surface were recorded, and where more than one species were hit with the one point, only the lowest hit was recorded.

Treatments and harvests

Experiment 1 comprised five treatments, replicated four times in randomised blocks. Each plot measured 20 links × 12.5 links. The area was mown on 7.11.57, and treatments applied on 8.11.57. All plots received a basal dressing of 200 lb per acre of double superphosphate, 200 lb gypsum, 100 lb muriate of potash, and 4 oz sodium molybdate. The following nitrogen treatments were applied: (1) no nitrogen; (2) no nitrogen but oversown with clovers, 2 lb per acre of each of the following: N.Z. white clover, alsike clover, N.Z. Montgomery red clover; (3) 50 lb N per acre; (4) 100 lb N per acre; and (5) 200 lb N per acre each, as "Nitrolime." All plots were oversown with cocksfoot (Grasslands Division, D.S.I.R., strain) at 10 lb/ac. Plots were sampled for flower stalk height by pulling out flowering tillers of browntop, sweet vernal, and Yorkshire fog on 13.2.58, and these tillers were dried and stored and later measured in the laboratory. Sample harvests were made as described earlier on 1.4.58, and the area mown and cleared. Top-dressings were re-applied as before on 12.9.58, and harvests made with mower on 20.1.59. Estimates of aftermath yields were made on 3.4.59. The area was cleared of herbage, and allowed to recover in 1959–60 without further application of manures. The final harvest was made on 19.3.60.

Experiment 2 comprised six manurial treatments, each one being replicated three times in a completely random design. Each plot measured 20 links × 25 links. The area was mown in early winter, and manures applied on 22.8.58. No further manures were applied. Harvests were made on 27.1.59 and 29.2.60. The manurial treatments were as follows: no manure, gypsum (150 lb/ac), urea (200 lb N/ac), urea and gypsum, "Nitrolime" (200 lb N/ac), "Nitrolime" and gypsum. No basal dressings were applied, and no oversowings were made.

Experiment 3 comprised 28 treatments. Three replicates were laid down in split plot design. Main plots consisted of no nitrogen and "Nitrolime" (200 lb N/ac). Sub plots were a 7 × 2 factorial represented by no sulphur, S25, S50, S100, S200 (each as sulphur), S25, and S100 (each as gypsum), all with and without phosphorus, at 150 lb per acre of double superphosphate. A basal dressing of sodium molybdate at 4 oz per acre was applied with an oversowing at the following rates per acre: cocksfoot 10 lb, N.Z. white clover 2 lb, alsike clover 2 lb, and N.Z. Montgomery red clover 2 lb.
from each plot.

RESULTS

Experiment 1

Table 2 presents values for flowerstalk height at 13.2.58 for the three principal adventive grasses on the Hogsback site. Insufficient samples of flowering tillers of fescue tussock were available after initial mowing to allow comparable measurements to be made of this species. Measurements were made of 40 sample tillers of each adventive grass from each plot.

TABLE 2. HEIGHTS OF FLOWER STALKS OF ADVENTIVE GRASSES 13.2.58
(in.)

Treatment No.	Nitrogen	Browntop	Sweet vernal	Yorkshire fog
1	N 0	12.5 d C	14.6 b B	16.5 c C
2	N 0 + Clover	13.0 cd C	14.8 b B	17.8 c C
3	N 50	14.5 bc BC	17.4 a AB	20.3 b B
4	N 100	15.4 b AB	18.1 a A	21.7 ab AB
5	N 200	17.2 a A	18.4 a A	23.1 a A
	s.e.	0.51	0.67	0.49

All three species showed marked responses in height to increments of nitrogen. Further unpublished data from these and other areas show a close relation between flower stalk height and tiller weight in each of these species.

Herbage yields at 1.4.58 are shown in Table 3. Only green material is included in the grass and clover fractions of this table. Only adventive grasses, variably composed of browntop, sweet vernal, and Yorkshire fog, showed significant increases with nitrogen applications.

It was obvious that plots varied considerably in botanical composition, and that these differences were not closely related to treatment. The principal variation in the harvested material in April 1958 was in the composition of the adventive grasses. Although volunteer white clover was present in many plots, in no plot did it yield more than

TABLE 3. HERBAGE YIELDS IN THREE SEASONS AT HOGSBACK
(lb per acre D.M.)

Treatment	Harvest 1.4.58			Harvest 20.1.59			Harvest 19.3.60		
	Grasses	Clovers	Total	Grasses	Clovers	Total	Grasses	Clovers	Total
N 0	760 c B	90 a	1,900 d C	740 d BC	1,110 b B	2,270 c B	2,140	1,380	3,630
N 0 + Clovers	930 bc B	180 a	2,490 cd BC	330 d C	5,010 a A	5,360 a A	740	7,780	8,520
N 50	1,280 bc B	70 a	2,790 bc BC	1,850 c B	830 b B	2,920 bc AB	1,430	790	2,280
N 100	1,580 b B	80 a	3,290 b B	3,840 b A	700 b B	4,810 ab AB	2,080	430	2,700
N 200	2,530 a A	10 a	4,330 a A	4,880 a A	60 b B	5,400 a A	1,270	40	1,860

200 lb DM per acre. This yield was approximately that of the oversown clovers in Treatment 2.

All plots were point analysed, as described earlier, after the area had been mown and cleared in April 1958. It was anticipated that these data would provide a suitable basis for co-variance analysis of yields of particular components during the following season, as well as indicate any changes in sward composition attributable to treatments. Results of this sward analysis showed the following features:

(i) An appreciable sward content of wild white clover on many plots which were not oversown with clovers, this resident clover being greatly stimulated by the application of the basal dressing.

(ii) Increased clover cover, following oversowing, at the expense of grasses and weeds.

(iii) A lack of any clear-cut effect of nitrogen treatments on sward composition, with the exception of a small increase in total grasses with high nitrogen levels.

After reapplication of treatments, including basal dressing, in spring 1958 harvests on 20.1.59 were considerably heavier than in the previous season, as is shown in Table 3.

Since no differences in ground cover of the four individual resident grass components were discovered in the previous April analysis, an analysis of co-variance for these components was done, adjusting for ground cover percentage. Results of this analysis, together with that for all vegetation, are presented in Table 4.

TABLE 4. YIELD OF SOME BOTANICAL COMPONENTS 20.1.59
(lb per acre D.M.)
(adjusted for ground cover percentage at autumn 1958)

Treatment		Browntop	Sweet vernal	Yorkshire fog	Native grasses	All vegetation
No.	Nitrogen					
1	N 0	750 b B	350 bc B	160 c C	330 a	2,640 c B
2	N 0 + Clover	530 b B	290 c B	210 c C	140 a	5,160 a A
3	N 50 N/Lime	880 b B	670 b B	520 b BC	350 a	3,350 bc AB
4	N 100 N/Lime	1,430 a A	630 b B	690 b B	580 a	4,690 ab AB
5	N 200 N/Lime	1,410 a A	1,150 a A	1,110 a A	780 a	5,580 a A

Considerable growth, estimated at up to 2,000 lb DM per acre, occurred on plots of treatments 2 and 5 after the harvest of 20.1.59, but no further harvests were made in that season. It was observed in April 1959 that, apart from the sown clovers of treatments 2, the evidence of autumn growth was confined principally to fescue tussock, and, in the case of treatment 5, sweet vernal.

The area was heavily grazed in patches by deer during the winter of 1959. No further application of manures was made, and the final

TABLE 5. HERBAGE YIELDS, 1958-9 AND 1959-60
(lb/acre D.M.)

Treatment	Browntop		Sweet vernal		All grasses		Dicotyledonous plants		Total	
	58/59	59/60	58/59	59/60	58/59	59/60	58/59	59/60	58/59	59/60
Nil	20 b B	110	20 b B	10 b C	50 c B	130	10 b BC	20	110 b B	160 e E
Urea (N 200)	140 b B	1,330	20 b B	40 b C	200 c B	1,370	10 b BC	0	290 b B	1,420 b B
N/Lime (N 200)	120 b B	620	40 b B	90 b BC	170 c B	700	2 b C	30	220 b B	800 d D
Gypsum (S 25)	30 b B	4	20 b B	10 b C	60 c B	50	20 b BC	20	120 b B	120 e E
U + S	450 a A	860	120 a A	490 a A	650 b A	1,390	70 a A	190	820 a A	1,690 a A
N/L + S	620 a A	540	160 a A	310 a AB	840 a A	870	60 a AB	150	980 a A	1,110 c C
Means										
Levels of gypsum										
S 0	90 a	690 a	30 a	50 a	140 a	730 a	10 a	20 B	200 a	800 a
S 25	370 a	480 b	10 a	270 a	520 a	770 a	50 a	120 A	640 a	970 a
Forms of nitrogen										
N 0	25 a	70 C	20 a	10 a	60 a	90 C	10 a	20 a	110 a	140 c
U (N 200)	290 a	1,100 A	70 a	270 a	430 a	1,380 A	40 a	100 a	550 a	1,560 a
N/L (N 200)	370 a	580 B	100 a	200 a	500 a	790 B	30 a	90 a	600 a	960 b
Interaction S × N	**	n.s.	**	**	**	n.s.	*	n.s.	**	**

harvest was taken, as described, during the autumn of 1960. Results are presented in Table 3.

When treatment 2, that oversown with clover, was tested against all others, it was found to be significantly lower in non-clover yield and highly significantly greater in both clover yield and total yield. No other differences were detected among nitrogen treatments. Non-clover yield in this 1959–60 season was found to be closely correlated with volunteer clover yield in 1958–9 from the plots on which clovers had not been oversown ($r = .609^{***}$). When non-clover yields in 1959–60 were corrected for clover yields in 1958–9, analysis of co-variance failed to show any differences among the non-oversown treatments.

Experiment 2

Table 5 presents results for the single harvests in each of the two seasons.

The single application of manures in 1958 had contrasting effects for the two seasons. Whereas in 1958–9 the nitrogen × sulphur interaction was significant for all harvest components, the addition of both sulphur and nitrogen being necessary for any significant increase in yield, in the second season browntop showed a highly significant increase with nitrogen but a significant decrease when gypsum had been added with nitrogen. Sweet vernal continued to exhibit the positive N × S interaction of the first season, while the dicotyledonous plants present in harvest, principally flat weeds with a trace of clover in the gypsum alone plots, showed so little response to nitrogen in the absence of sulphur that only the sulphur treatment was significant.

Experiment 3

Table 6 presents a summary of results for the single harvests in each of two seasons. The values for S are means of all applied sulphur levels and forms, although response from 25 lb elemental sulphur was generally intermediate to no sulphur and high levels.

The native grasses, almost entirely fescue tussock, responded to additions of sulphur, phosphorus, and nitrogen together, but were highly significantly lower in yield when only nitrogen was applied. In the second year they were very low in comparison with the first year, and no significant responses were measured.

Adventive grasses showed a fivefold response in the first year to nitrogen alone, which was further increased threefold by the addition of sulphur and phosphate together. In the second year the adventive grasses showed a twofold response to nitrogen, which was further increased in the presence of added phosphate. As in the second year of Experiment 2, the yield of adventive grasses on plots receiving only nitrogen was almost entirely browntop. When sulphur or phosphate was applied with the nitrogen, sweet vernal was an appreciable additional component. Yorkshire fog was a minor component of yield on some plots, especially those receiving all three added nutrients.

TABLE 6. HERBAGE YIELDS, ENYS FLAT
(lb per acre D.M.)

Treatment	1958–9			1959–60		
	Native grasses	Adventive grasses	Total vegetation	Native grasses	Adventive grasses	Total vegetation
Nil	200	10	210	10	270	290
S	140	30	170	10	210	260
P	110	20	130	20	290	320
SP	160	20	190	10	220	250
N	60	140	190	0	520	520
NS	220	130	360	20	740	790
NP	120	60	180	10	1,020	1,110
NSP	450	400	860	30	960	1,030
Significant effects						
N	**	**	**	n.s.	*	*
N × P	**	*	**	n.s.	**	**
N × P × S	**	**	**	n.s.	n.s.	n.s.

Dicotyledonous plants, principally *Hypochaeris radicata, Hieracium praealtum* var. *decipiens,* and *Rumex acetosella* were generally responsive to nitrogen and phosphate, but their yield was small in comparison with the grasses.

DISCUSSION

Three principal features of herbage growth under these experimental conditions have been illuminated by the results obtained. These are: (1) the influence of site and of level of applied nitrogen on the level of herbage production; (2) the influence of other nutrients on the effectiveness of applied nitrogen; and (3) the differential influence of manurial treatment and management on the botanical composition of yield. These three features are discussed in turn.

Influence of site and level of applied nitrogen on herbage yield

Resident herbage yields in response to dressings of 200 lb N on Craigieburn soils were generally about 1,000 lb DM per acre, provided other nutrients were not limiting. This level of production represents an increase over no manure of from five to 10 times, depending on the actual level of production of the control plots. In the case of the deeper, more moist, and more fertile soil of the Hogsback site, the total herbage production in comparable periods with similar additions of nitrogen was approximately 5,000 lb DM per acre, about two and a half times

the no nitrogen level. The yield of grass herbage from 200 lb N per acre on the Hogsback site was increased about fivefold, as on the Craigieburn soil. The yield of grass in the 1958–9 season on the Hogsback site showed a linear relation to increments of nitrogen as "Nitrolime," according to the equation

$$G = 21.16N + 976$$

(standard error of regression coefficient = 4.16),

where G is yield of grass in lb D.M. per acre and N is annual application of "Nitrolime" in lb of nitrogen per acre. No comparison of levels of nitrogen has been made on uncultivated pastures on the Craigieburn soil.

The general picture presented from the experiments on both soils is that the resident grasses are increased in yield by an amount equivalent to their pre-treatment yield for every increment of 40 lb of fertiliser nitrogen.

Influence of sulphur and phosphate on the effectiveness of applied nitrogen

Phosphate and sulphur have made possible a fuller response by grasses to applied nitrogen. In the first year of Experiment 1 and in all harvests of Experiments 2 and 3, clover production had been nil or of very small magnitude. There is little doubt, therefore, that the influence of sulphur on responses to nitrogen recorded from Experiment 2, and similarly that of phosphate and sulphur from Experiment 3, are direct effects on the adventive and native grasses, and are not attributable to augmented nitrogen as an outcome of improved clover growth. Morrison (1958) found in pot experiments with Tekoa soil, slightly inferior in citric-soluble phosphate to the Craigieburn soil, that ammonium nitrate by itself or in combination with potassium or potassium plus sulphur, had little effect on the growth of fescue tussock, whereas, with phosphate, responses to nitrogen were large. The present work indicates that browntop in particular may be less demanding for, or superior in obtaining, phosphate and sulphur than is fescue tussock.

Influence of manurial treatments and management on botanical composition

Marked treatment differences in botanical composition of harvests were shown in all experiments. On land of higher native fertility the increases in height and yield with successive increments of nitrogen are indicated in Tables 2 and 4 to be greater for Yorkshire fog than for sweet vernal and browntop, and even more so than for native grasses. It is concluded therefore that improvement in available nitrogen under conditions of adequate sulphur and phosphate would tend to result in considerable increase in the Yorkshire fog component of both swards and harvests. A similar trend is indicated on the Craigieburn soils of lower native fertility by the evidence of Yorkshire fog in plots receiving sulphur, nitrogen, and phosphate together. Where nitrogen was applied on the Craigieburn soils with correction of phosphate or sulphur deficiency, the composition of the harvest was greatly different from that on plots receiving nitrogen alone, especially in respect to the balance of sweet vernal and browntop. Improvement in

nitrogen supply without concomitant improvement in the supply of sulphur resulted in an almost exclusively browntop harvest. Sulphur was not necessary to obtain a browntop yield response from added nitrogen in the second year after application. A superior competitive ability or lower need for scarce sulphur is shown by browntop over sweet vernal in manner similar to that of sweet vernal over Yorkshire fog shown by Vartha (1960) in a somewhat similar tussock grassland area. In view of the suggested role of cation exchange capacity of roots of different species in this competition for sulphur, as outlined by Jackman (1960), it would appear that the apparently similar C.E.C. properties of browntop and sweet vernal warrant closer investigation.

It is noteworthy also that the native grasses were prominent in response to fertiliser only in Experiment 3 which did not involve pre-cutting. Once the plots had been subjected to mowing, the growth of fescue tussock tended to be greatly reduced below that of adventive grasses. Unpublished data from other experiments involving hand clipping suggest that repeated defoliations of fescue tussock greatly weaken its competitive ability with other resident grasses.

Where, as illustrated in Table 3, clovers of more vigorous strains are introduced under conditions of adequate nutrition, their growth may be such as to reduce the contemporary yield of resident grasses under certain conditions of defoliation. A similar phenomenon has been shown on cultivated pastures in an earlier paper in this series, where additional supply of nutrients in a basal dressing resulted in a major increase in clover production and a small but barely significant decrease in grass growth.

CONCLUSION

The resident grasses of the uncultivated pastures are clearly shown to be suffering from serious nutritional deficiencies. These deficiencies are not confined to nitrogen but include sulphur and phosphate. When poor clovers are stimulated by manuring there is an improvement in subsequent grass growth. When clovers of high inherent vigour are adequately manured they can be expected to suppress resident grass growth, especially under infrequent defoliation. Even on soils of high natural fertility with close C:N ratio this suppression may last for some years.

The responsiveness of grasses at Hogsback to high levels of nitrogen supply, and the relatively low level of grass production at Enys Flat, even at the high level of nitrogen application, indicate the large improvement in soil nitrogen that must be made before even resident grasses can produce to the capacity of the climate.

These two features emphasise the importance of management of clover-oversown pastures for clover dominance for a number of years. If the clover-dominant phase in pastoral development is truncated by failure to maintain fully adequate supplies of sulphur and phosphate then a poor clover-browntop association may be expected, declining further in clover with further neglect of manuring. If, however, the

manurial programme is maintained for long-term clover dominance until a much higher nitrogen and other nutrient regime is reached, it may be expected that an association will develop consisting chiefly of clovers, sweet vernal, and Yorkshire fog, as well as other invading or oversown "high fertility" grasses, such as ryegrass and cocksfoot.

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REFERENCES

Dingwall, A. R. 1956: Tussock grassland research in the Broken River area. *N.Z. J. Agric. 93:* 277–83.

Jackman, R. H. 1960: Competition between pasture species for nutrients from the soil. In "1960 Sheepfarming Annual." pp. 75–84. Massey Agricultural College, Palmerston North.

Lobb, W. R. 1958: The improvement of foothill country. *Proc. 20th Conf. N.Z. Grassl. Ass.* pp. 50–64.

Lobb, W. R.; Bennetts, R. L. 1958: Improvement of tussock grassland in Canterbury. *N.Z. J. Agric. 96:* 537–49.

Morrison, T. M. 1958: Biological studies of some tussock-grassland soils. XI. The response of a tussock plant, *Festuca novae-zelandiae,* to fertilisers. *N.Z. J. agric. Res. 1:* 1003–5.

O'Connor, K. F. 1959: Plant production in difficult environments. *Proc. N.Z. Inst. Agric. Sci. 5:* 86–96.,

————1961a: Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. I. *N.Z. J. agric. Res. 4:* 686–97.

————1961b: Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. II. *Ibid. 4:* 698–708.

Vartha, E. W. 1960: "Grass nutrition: sulphur." pp. 44, 61. Thesis deposited in Canterbury Agricultural College Library. 98 pp.

Walker, T. W.; Adams, A. F. R. 1958: Competition for sulphur in a grass-clover association. *Plant & Soil 9:* 353–66.

Walker, T. W.; Adams, A. F. R.; Orchiston, H. D. 1955: The effects and interactions of sulphur, phosphorus and molybdenum on the growth and composition of clovers. *N.Z. J. Sci. Tech. A36:* 470–82.

————1956: The effect of levels of calcium sulphate on the yield and composition of a grass and clover picture. *Plant & Soil 7:* 290–300.

SHORT COMMUNICATION

PHYTOTOXIC PROPERTIES OF SOME HYDROLYTIC PRODUCTS OF SPORIDESMOLIDE I

(A note)

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ABSTRACT

Solutions of sporidesmolic acids A and B inhibited the 2, 4-D-stimulated elongation of barley coleoptile sections, and caused symptoms of toxic wilting in detached *Chenopodium* leaves. They did not affect the germination of barley and cress seeds, and the seedlings developed normally in contact with the solutions.

Two metabolic products of the pasture fungus *Pithomyces chartarum* (Berk. & Curt.), M. B. Ellis have been investigated chemically. One is the powerful hepatotoxin sporidesmin (Synge and White 1959), the other the cyclic depsipeptide (Schemjakin 1960) sporidesmolide I (Russell 1960, 1962). The latter, a highly insoluble compound, is hydrolysed under mildly alkaline conditions to the soluble sporidesmolic acids A (Russell 1960) and B (Russell and Brown 1960). These acids, together with sporidesmolide I, may thus be

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present in the soil and litter of pastures which from time to time support vigorous growth of the fungus (Russell *et al.* 1962). In view of the phytotoxic properties of the compounds structurally related to sporidesmolide I and to the sporidesmolic acids (Curtis 1961; Woolley *et al.* 1955; Clausen-Kaas *et al.* 1944), investigation of the effects of the *P. chartarum* metabolite and its degradation products was undertaken.

In preliminary experiments, petioles of three detached leaves of *Chenopodium amaranticolor* L. were immersed in a 10^{-2}M solution of sodium sporidesmolate B. Their subsequent water uptake was markedly depressed in comparison with that of control leaves whose petioles were immersed in distilled water or in a 10^{-2}M solution of sodium acetate. After 24 h the treated leaves were showing marked and progressive chlorosis of the interveinal areas, and two were obviously wilted; the third was flaccid, and wilted later. None of the treated leaves recovered when transferred to distilled water. The control leaves retained a normal appearance throughout the experiment (36 h). Similar results were obtained at a concentration of 3×10^{-3}M with sodium sporidesmolate A and with sodium sporidesmolate B. The visible effects were similar to those produced in tomato cuttings by lycomarasmin (Gäumann and Jaag 1946, 1947).

The toxicity of sporidesmolic acids A and B was studied more quantitatively by the barley coleoptile section test (Ludwig *et al.* 1957) which measures the inhibition, by the substance under test, of the 2, 4–D-stimulated elongation of the sections. For sodium sporidesmolate A, the $E.D._{50}$ was found to be 6.5×10^{-3}M, and for sodium sporidesmolate B, 2.3×10^{-3}M. These values may be compared to that for maleic hydrazide, viz. 1.7×10^{-3}M (Ludwig *et al. loc. cit.*). Only feeble inhibition was produced by a solution prepared by shaking the total sporidesmolide fraction (Done *et al.* 1961) of *P. chartarum* with water for one week and filtering. Lycomarasmin appeared to be non-toxic in this test, but this could have been due to its instability in aqueous solution (E. Gäumann, pers. comm.).

Clear evidence was thus found for a toxic action of sporidesmolic acids A and B on isolated plant structures. Their effect upon whole plants was therefore next studied. When cress seeds were placed on filter papers moistened with solutions of sodium sporidesmolate A (10^{-2}M) or B (3×10^{-3}M), or impregnated with the total sporidesmolide fraction (10 mg) and moistened with water, no effect upon germination or root growth could be demonstrated. Similarly, barley seeds germinated normally following planting in pumice and watering with sodium sporidesmolate A or B solutions of the concentrations cited, and no effect upon the length of coleoptiles or of leaves was observed. There is thus no evidence for a toxic effect of these substances upon intact plants.

P. chartarum is believed to be an obligate saprophyte (Miss J. Dingley, pers. comm.), and its hyphae have not been observed to invade living plant tissues. Thus the phytotoxicity of the sporidesmolic acids is unlikely to possess any significance in the field.

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REFERENCES

Clausen-Kaas, N.; Plattner, P. A.; Gaumann, E. 1944: Uber ein welkerzeugendes Stoffwechselprodukt von *Fusarium lycopersici* Sacc. *Ber. schweiz. bot. Ges. 54:* 523–8.

Curtis, R. W. 1961: Studies on response of bean seedlings and corn roots to malformin. *Plant Physiol. 36:* 37–43.

Done, J.; Mortimer, P. J.; Taylor, A.; Russell, D. W. (1961): Production of sporidesmin and sporidesmolides by *Pithomyces chartarum*. *J. gen. Microbiol. 26:* 207–22.

Gaumann, E.; Jaag, O. 1946: Uber das problem der welkekrankheiten bei pflanzen. *Experientia 2:* 215–20.

————1947: Die physiologischen grundlagen des parasitogenen Welkens I–III. *Ber. schweiz. bot. Ges. 57:* 3–34; 132–48; 227–41.

Ludwig, R. A.; White, R. W.; Unwin, C. H. 1957: A rapid quantitative test for phytotoxicity and its use in the examination of a series of alkyl-substituted cyclohexenones. *Canad. J. Bot. 35:* 605–13.

Russell, D. W. 1960: Sporidesmolide I, a metabolic product of *Sporidesmium bakeri* Syd. *Biochim. biophys. Acta 45:* 412–3.

————1962: Depsipeptides of *Pithomyces chartarum*: the structure of sporidesmolide I. *J. chem. Soc.:* 753–61.

Russell, D. W.; Brown, M. E. 1960: Sporidesmolic acid B, an hydroxyacyl dipeptide from *Sporidesmium bakeri*. *Biochim. biophys. Acta. 38:* 382–3.

Russell, D. W.; Synge, R. L. M.; Taylor, A.; White, E. P. 1962: Similarity of depsipeptides from *Pithomyces chartarum* and from pasture samples from 'facial eczema' areas. *J. chem. Soc.:* 554–5.

Schemjakin, M. M. 1960: Der chemie der depsipeptide. *Agnew. Chem. 72:* 342–5.

Synge, R. L. M.; White, E. P. 1959: Sporidesmin: a substance from *Sporidesmium bakeri* causing lesions characteristic of facial eczema. *Chem. & Ind. (Rev.):* 1546–7.

Woolley, D. W.; Schaffner, G.; Braun, A. C. 1955: Studies on the structure of the phytopathogenic toxin of *Pseudomonas tabaci*. *J. biol. Chem. 215:* 485–92.

TECHNOLOGY

COLLECTION AND CONCENTRATION OF SPORES OF *Pithomyces chartarum* FROM HERBAGE AND HARVESTING FROM RYECORN CULTURES

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ABSTRACT

A description is given of the techniques for the collection and concentration of spores (conidia) of *P. chartarum* from grassland herbage, for chemical and biological assays for the toxin sporidesmin, which is implicated in facial eczema of sheep and cattle.

A metal gauze and cotton wool filter system or a cyclone, both powered by an electric suction cleaner, are used for the collection of spores mixed with fine debris from herbage. The spores of *P. chartarum* are concentrated by rolling spores and debris on typing parchment to which the spores adhere, or by suction through fine nylon fabric with small glass cyclones; dry harvesting of spores grown on ryecorn is done with a cyclone. A wet spore harvesting technique is described.

The field-cyclone technique may be used for "wet collection" from herbage for assessment of spore load when spore traps cannot operate and for collection of small insects and other fungi.

INTRODUCTION

Pithomyces chartarum (Berk. & Curt.), M. B. Ellis, the fungus implicated in facial eczema of sheep and cattle (see Smith *et al.* 1961) grows and sporulates freely, producing a relatively small proportion of mycelium to spores on litter in herbage under moist warm conditions. In dry weather, following warm moist periods, spores (conidia) may be found in abundance on living green leafage, selected (in preference to dead material) and ingestd by sheep which subsequently may develop symptoms of facial eczema. These spores have not been noted to germinate, and so do not produce mycelium on living green leaves.

It was found by Synge and White (1959) that spores (and mycelium) of *P. chartarum* (synonym *Sporidesmium bakeri*, Syd.) contain the toxin sporidesmin, which causes the typical liver lesions of

facial eczema in guinea-pigs and has been shown by Mortimer and Taylor (in press) to produce these symptoms in sheep. Most isolates of the fungus from the field show pleomorphism and variation in spore production in culture. Chemical assays for sporidesmin content by the azide-iodine method (Russell 1960; Clare in press) of first subcultures of 6 field isolates grown under standard conditions on ryecorn (Lloyd and Clarke 1959) showed a variation in toxin content of 0.08% to 0.12% (G. R. Russell pers. comm.). Chemical assays for sporidesmin in the spores attached to harvested plant material is made difficult (particularly for routine work) by interfering plant substances (N. T. Clare pers. comm.).

Thornton and Sinclair (1960) found a relation between relatively high counts of spores obtained by a "wash and haemocytometer count" technique on cut herbage and facial eczema. Smith *et al.* (in press) found a broad relation between estimated mobile spore-trap counts (Brook 1959) and the severity of facial eczema in lambs. Anomalies occurred where very high spore counts were associated with moderately severe facial eczema and relatively low counts were associated with severe facial eczema.

It is most probable that the spores of the fungus are the main source of toxin to sheep. As the severity of facial eczema may be related to both spore load on herbage and to spore toxicity, techniques were required for the collection of spores from field herbage in sufficient quantities for chemical and biological assays for toxicity.

Fig. 1.—Filter chamber, filters, and material collected.

It is convenient and satisfactory in the production of sporidesmin for chemical studies and experiments with animals to extract whole cultures of *P. chartarum* on ryecorn with solvent. It is necessary also, in some cases, to have preparations of spores without the substrate for chemical and biological studies. Techniques were devised for this purpose.

MATERIALS AND METHODS

Collection from herbage

1. *Filter-chamber method*

A filter chamber (Fig. 1) is fitted between one of the rigid tubes and the fan-shaped nozzle of a large domestic vacuum cleaner of the cylindrical type. The chamber is made from 20-gauge sheet copper and 1¼ in. external diameter brass tubing. It consists of two cups, one of which fits inside the other with 1/32 in. clearance. The outer shell is 2½ in. external diameter and 1½ in. long. A rubber washer is cemented inside the base of the inner chamber. Two filter holders and a collar ¾ in. deep, all cut from stout brass tubing 2¼ in. in diameter, fit inside the inner chamber. The filters are made by soldering brass gauze, of 36 and 50 meshes per inch respectively, inside the holders. A rubber washer identical to that in the filter chamber is cemented to one edge of each filter holder and the collar.

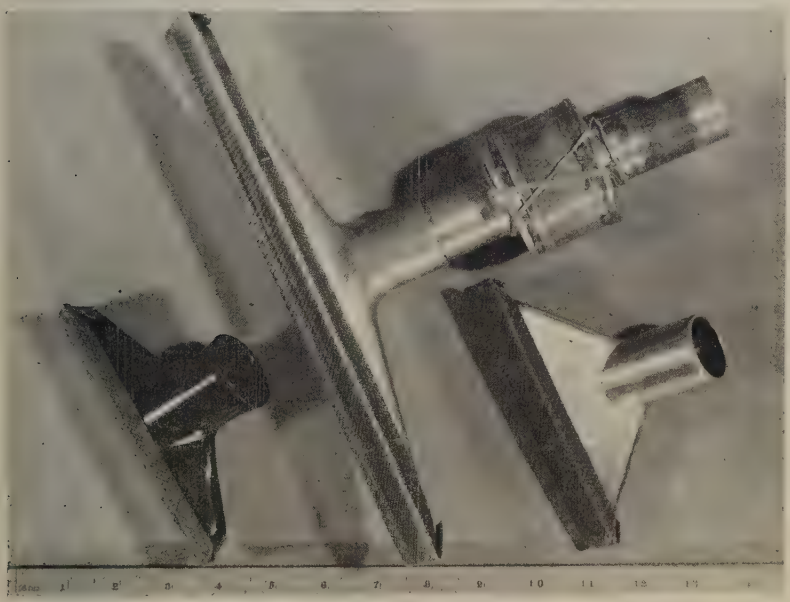

Fig. 2.—Fan-shaped suction nozzles with gauzes and assembled filter system.

A piece of 36-mesh gauze is securely folded over the outside of a fan-shaped suction-nozzle (Fig. 2), and the latter is fitted to the 1¼ in. brass tube connection of the inner filter chamber. The 50-mesh filter is placed in the inner chamber, metal edge down; this is followed by the metal collar, also metal edge down, which is packed loosely but completely with absorbent cotton wool. The 36-mesh filter is placed over the collar and the chamber closed with the outer casing, which is secured to the inner one with strong rubber bands. The chamber is then attached by the brass tube to the rigid tube of the vacuum cleaner.

For use in the field, a mains-isolation transformer is recommended, even if the suction-cleaner motor armature is double-wound for mains isolation. With the power on, the nozzle is drawn firmly over dry herbage known to harbour the required fungus in large numbers. The gauze on the nozzle filters out the larger pieces of debris from plants and soil. The 50-mesh filter holds back the smaller pieces of material, which impinge and accumulate on its outer surface, forming, in effect, a fine filter system. Fungal spores collect on the debris on this filter, but some pass through and collect on and penetrate into the cotton wool packed in the collar (Fig. 1). The 36-mesh filter behind this prevents the cotton wool from being drawn into the suction tube. Filters of finer mesh in this position restrict unduly the air flow through the system. Spores are concentrated from the debris as described below under "Spore concentration from litter".

2. *Cyclone method*

Small cyclones for the collection of the spores of cereal rusts and other fungi from leaves have been described by Tervet *et al.* (1951); larger cyclones are in common use in commercial processes for particle collection. The cyclone equipment shown diagramatically in Fig. 3 consists of a spun copper upper portion and a glass collection vessel (quart preserving jar) which is screwed to a flange soft-soldered to the base of the metal chamber. A compression washer fitted between jar and cyclone body provides an airtight joint. A brass intake-tube is brazed tangentially to the side of the upper portion of the metal part of the cyclone, and a similar suction outlet tube is brazed centrally in the head. The inner portion of this latter tube is positioned slightly below the level of the tangential intake tube. Dimensions of the equipment are shown in Fig. 3.

A high-efficiency suction cleaner is attached to the suction outlet tube of the cyclone via plated brass tubing, reinforced flexible plastic hose, short articulating tube, and rubber sleeve. Suction heads with metal gauzes of the type described in Method 1 (Filter-chamber method) are attached to the intake tube of the cyclone via metal tubing, reinforced plastic hose, and closely-machined thick-walled Perspex sleeving to provide an air-tight fitting. Metal to hose joints are cemented with epoxy-resin glue.

This equipment may be used on dry herbage for the collection of litter and spores in the same way as in Method 1 and the spores

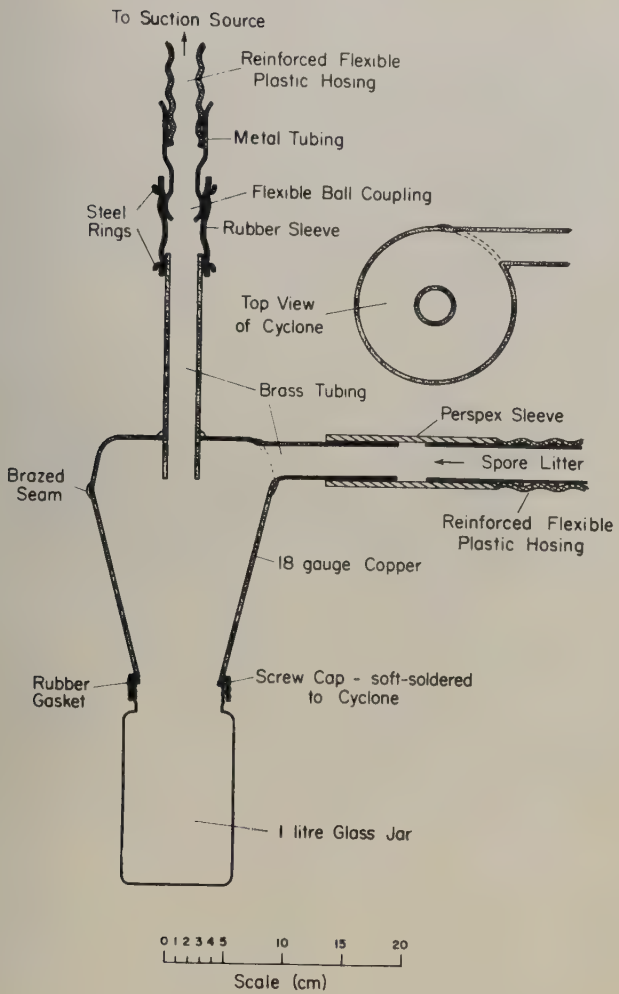

Fig. 3.—Cyclone for field collection of spores (in section).

separated from the litter. (See below.) It can also be used in "wet-pick-up" procedures on herbage when it is wet with dew or rain. (See Discussion.) Under wet conditions, stainless steel suction-nozzle extension tubing is used.

Spore concentration from litter

In the filter-chamber method of collection, fine debris impinges on the surface of the 50-mesh filter. Some passes through it, and is shaken off the lower surface of the cotton wool. Both collections may contain

a high proportion of conidia of dry-spored fungi, *P. chartarum, Epicoccum* sp., *Helminthosporium* spp., and *Puccinia* sp. Small insects and mites in considerable numbers are also frequently collected. To concentrate the spores of *P. Chartarum* the following methods may be used:—

(a) *Dry paper method*

The collected material is rolled back and forward several times on typing parchment over a tray. The excess material is tipped off, and the paper shaken vigorously. The adhering spores may then be brushed up with a soft wide brush and collected. The product consists of a large proportion of conidia of *P. chartarum,* a smaller proportion of other fungal spores, and small fragments of siliceous material.

(b) *Oiled paper method*

A drop of liquid paraffin is placed on a large plug of absorbent cotton wool. The plug is folded over, and firmly stroked over the typing parchment. The collected material is then added to the paper as mentioned above under (a). The total recovery of spores by this "oiled paper" method is higher than in the "dry paper" technique, but a greater proportion of conidia of *Epicoccum* sp. and *Helminthosporium* spp. and fine siliceous material is collected.

(c) *Cyclone separation*

The equipment consists of two glass cyclones, illustrated in Fig. 4, set up in bench clamps. The first-stage cyclone is made from a conical flask fitted with detachable collection tube connected to its base with a B19 ground glass joint and rubber bands. The second cyclone, which is smaller but in other respects similar, is connected in series with the first, and deposits finer particles and acts as a trap to prevent fine dust reaching the suction pump and venting into the atmosphere.

A suction-nozzle is prepared by obliquely grinding the end of a short piece of 5 mm Pyrex glass tubing and fusing the ground surface. This is connected to the intake of the first cyclone with clear P.V.C.* tubing. The cyclones are powered by a $\frac{1}{4}$ h.p. electric motor and rotary pump, giving a negative pressure of 10 lb per sq. in.

Cotton wool plugs containing spores collected by the filter-chamber method are carefully spread out on a sheet of plate glass. Fine debris, with spores collected by the latter method or in the field cyclone, is spread thinly in large petri-dish lids. The spore-bearing material is covered with a piece of fine nylon fabric** which is

* This should not be rubber tubing as the azide-iodine assay used in the estimation of sporidesmin in spores is based on a sulphur reaction.

**This may be fine-mesh knitted nylon hosiery material or bolting nylon. It may be folded to form a finer filter system. Surgical gauze or similar fluffy material is not suitable.

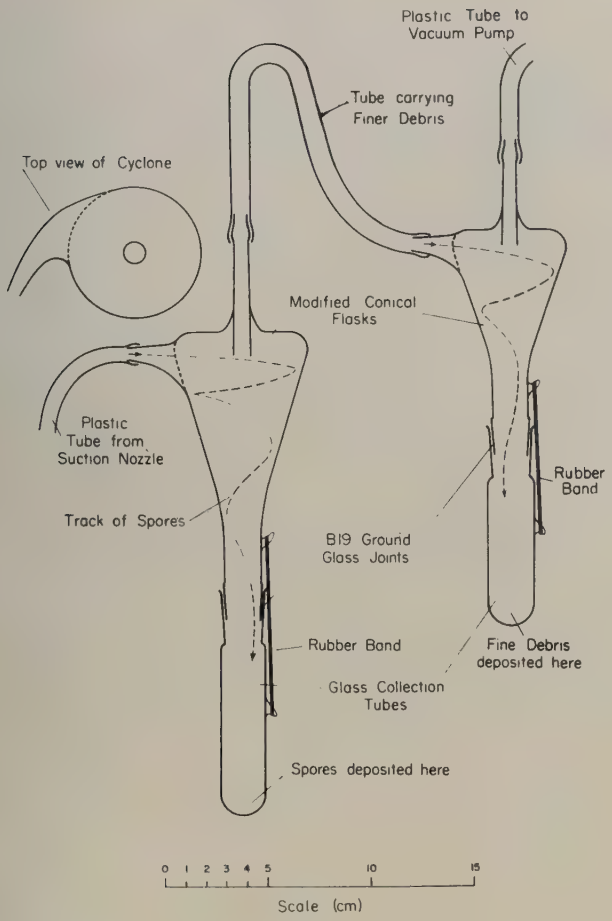

Fig. 4.—Small laboratory glass cyclones for separation and harvesting of spores (in section).

stretched taut. The suction-nozzle connected to the first cyclone is drawn over the surface of the pad. The spores "cyclone" down and collect in the detachable tube of the first cyclone. The spore-bearing material may be turned over, covered again with nylon filter fabric, and re-treated.

Harvesting spores from cultures

(a) *Dry harvesting*

Cultures on agar media or on ryecorn are dried off at room temperature or in an oven at a temperature not exceeding 35°c for

1 hour (if the spores are required for germination studies). Higher drying temperatures (up to 70°c for 1 to 2 hours) may be employed if the sporidesmin is to be extracted with solvents.

Spores may be removed from dried agar cultures or ryecorn cultures by covering these with nylon filter material. The spores are then harvested with the cyclone.

(b) *Wet harvesting*

At the end of the incubation period ryecorn cultures of the fungus in flasks or bottles are just covered with a 0.1% solution of wetting agent, capped with polythene, and shaken until the contents are "wetted". The whole contents are emptied into large beakers and stirred gently for 10 min. with an electric stirrer. The liquid, containing spores, is filtered first through 14-mesh wire gauze and then through close-woven Terylene cloth in Buchner funnels under suction. As much moisture as possible is removed by filtration from the cake of spores, which is then broken up, spread on trays, and dried.

Safety precautions for handling dry spores

The toxic hazards to humans of the spores of *P. chartarum* are not known. P. H. Mortimer (pers. comm.) has found that severe lesions can result from the instillation of spores into the eyes of experimental animals. When spores are being handled (particularly in bulk and when dry) suitable eye shields and respirators*** should be worn. Laboratory cyclones for spore-harvesting should be operated in a suitable force-ventilated hood, with the vent protected by a filter system to cointain scavenged spores.

DISCUSSION

The techniques and equipment described were principally designed for the collection and concentration of the spores of *P. chartarum* (and other fungi) from grassland herbage and for spore harvesting for chemical and biological assays for sporidesmin. These assays are proceeding, and the results will be reported in due course. The field-cyclone technique may be used for the collection of small insects and other fungi.

The cyclone collection technique can be used to pick up spores in moisture when herbage is wet with dew or rain. It is possible to use this method as a rapid means of assessing spore load on herbage when the volumetric spore trap cannot be used.

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***Clear plastic eye-shields and plastic half-respirators with fine-dust filters.

REFERENCES

Brook, P. J. 1959: *N.Z. J. agric. Res. 2:* 690–3.

Clare, N. T. (in press): *Symp. Facial Eczema, N.Z. Microbiol. Soc.*

Lloyd, A. A.; Clarke, R. T. J. 1959: *N.Z. J. agric. Res. 2:* 1084.

Mortimer, P. H.; Taylor, A. (in press): *Res. vet. Sci.*

Russell, G. R. 1960: *Nature, Lond.*: 186, 788–9.

Smith, J. Drew; Crawley, W. E.; Lees, F. T. 1961: *N.Z. J. agric. Res. 4:* 538–51.

————(in press): *Symp. Facial Eczema, N.Z. Microbiol. Soc.*

Synge, R. L. M.; White, E. P. 1959: *Chem. & Ind. (Rev.) 49:* 1546.

Tervet, I. W.; Rawson, A. J.; Cherry, E.; Saxon, R. B. 1951: *Phytopath. 41:* 282–5.

Thornton, R. H.; Sinclair, D. P. 1960: *N.Z. J. agric. Res. 3:* 300–13.

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